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SIXTH TECHNICAL ALFALFA CONFERENCE

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Held at Albany, California
July 16, 1959

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Sponsored by Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany, California, and American Dehydrators Association, Kansas City, Missouri.

Agricultural Research Service
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THE SIXTH TECHNICAL ALFALFA CONFERENCE sponsored by the Western Utilization Research and Development Division of USDA's Agricultural Research Service and the American Dehydrators Association was held in Albany, California, July 16, 1959.

The program was arranged by George O. Kohler, Acting Chief of the Field Crops Laboratory, Western Utilization Research and Development Division; and Joseph Chrisman, Executive Vice President of the American Dehydrators Association.

The sponsors welcomed 63 participating scientists, alfalfa dehydrators, equipment manufacturers and others interested in alfalfa and forage production and processing from 17 States, Washington, D. C., and British Columbia.

This publication is a report of information presented at the Conference and was prepared for the permanent records of the participants and others who may desire it. Copies are available on request from the Western Regional Research Laboratory, U.S.D.A., Albany 10, California.

Morning Session

George O. Kohler, Moderator

Vitamin and Chemical Studies in Dehydrated Forages	Dr. H. P. Binger Western Regional Research Laboratory
Quality Control in Forage Harvesting and Processing	Mr. Robert Fulton Archer-Daniels-Midland Company Kansas City, Missouri
Farm Research on Alfalfa	Dr. C. H. Hanson Crops Research Division A.R.S., U.S.D.A. Beltsville, Maryland
Engineering Aspects of Hay Wafering and Pelleting	Mr. J. B. Dobie University of California Davis, California

Afternoon Session

Joseph Chrisman, Moderator

Review of ADA Research Program	Mr. Joseph Chrisman American Dehydrators Association Kansas City 6, Missouri
Use of Pelleted and Other Forms of Alfalfa in Ruminant Feeding	Dr. J. H. Meyer University of California Davis, California
Review of Grass Juice Investigations	Dr. G. O. Kohler Western Regional Research Laboratory
Forage Juice in Swine Feeds	Dr. D. I. Gard Eli Lilly & Company Greenfield, Indiana
The Chemistry and Distribution of Estrogens in Forages	Mr. E. M. Bickoff Western Regional Research Laboratory
The Past, Present and Future of Estrogens in Feeds	Dr. A. N. Booth Western Regional Research Laboratory
Where We Stand on Saponins	Dr. C. R. Thompson Western Regional Research Laboratory

VITAMIN AND CHEMICAL STUDIES IN DEHYDRATED FORAGES

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There exists a constant demand for information concerning forage constituents, especially with regard to variation with stage of growth, and to alterations brought about by processing practices, for example, pelleting and regrinding. Many workers have reported increased weight gains when ruminants were fed pellets as opposed to dehydrated meals. The cause of this apparent growth stimulation is not clear, and this is one of the factors which prompted our study. Through the years a great quantity of compositional data has been reported, as may be seen, for example, in the recent compilation by the National Research Council, the result of a project supported by the Department of Agriculture. Unfortunately, however, most of the data which are available from all sources do not permit adequate correlations because different constituents have been determined in different samples of a forage. In this project, therefore, we decided to select a limited number of samples and to study these as intensively as our resources permitted.

We are indebted to the members of the American Dehydrators Association, and especially to its executive vice president, Mr. Joseph Chrisman, for assistance in obtaining the samples used. In addition, the vitamin assays were performed by the Wisconsin Alumni Research Foundation under the terms of a Research and Marketing Act contract between the Foundation and the Agricultural Research Service of the U. S. Department of Agriculture.

Commercially processed forage samples were obtained from a wide geographical area. Of the total of 24 samples, 18 were alfalfa (12 meals and 6 reground pellets), while 4 were grasses and 2 were grass-legume mixtures, all as dehydrated meals. A fairly complete agronomic history was obtained for each of the samples used in this study.

The ultimate objective of forage analysis is to judge its value as a feed ingredient. Because most laboratories are unable to carry out actual feeding trials, and because these are lengthy and expensive, chemical analysis is regularly used for feed evaluation. Additionally, feeding experiments yield data in the nature of gross resultants of many factors. These data are desirable for judging the over-all feeding value of a sample, but they do not provide the specific information required for other purposes, such as investigations of the effects of processing on specific plant constituents.

In our study, chemical analyses were carried out according to the scheme of "proximate analysis," and also by what may be called "summative analysis." We are therefore able to make certain comparisons which may be useful for estimating the relative values of some of these analytical procedures.

Thirteen fat-soluble and water-soluble vitamins and accessory factors were determined in all 24 samples. Because of extended storage prior to assay, the data for some of the labile constituents, for example, carotene and xanthophylls are not reliable. The only significant vitamin losses which resulted from pelleting and regrinding alfalfa, were of inositol (about 25% loss) and pyridoxine (about 30% loss). In general, younger plant material, higher in protein, was also higher in vitamin content.

Data for other consitutents show the same general trend as the vitamin data; that is, younger plant material contains more nutrients and less fiber or cell wall material. These data also indicate that it may be possible to estimate both total cell wall material, and lignin content of a forage merely by performing two successive extractions (with ethanol-benzene azeotrope and ammonium oxalate) followed by determination of nitrogen in the residue. The only effect of the pelleting and regrinding process on chemical constituents other than vitamins, was an apparent increase in crude fat (ethyl ether extractives) in the reground pelleted alfalfa samples when compared with the dehydrated meals. This may possibly be attributed to rupture of the chloroplasts resulting from the heat and pressure generated in the pelleting operation.

The grasses and grass-legume mixtures studied were found to be excellent sources of most nutritive factors, being high in their contents of protein, fat, free sugars, and most vitamins, while containing only moderate amounts of fibrous cell wall material. Most of these attributes, however, were matched by the best alfalfa samples analyzed, i.e., those with approximately 25% protein content.

As far as dehydrated alfalfa is concerned, the chemical alterations caused by pelleting and regrinding do not appear to be sufficient to account for a growth increase. We must therefore assume that the dominant factor in increased weight gain is the higher bulk density of the pelleted materials.

Complete details of procedures and data are expected to be published shortly in the form of a Technical Bulletin.

QUALITY CONTROL IN FORAGE HARVESTING AND PROCESSING

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Any discussion of quality control in the harvesting and processing of forage crops must take into consideration the progress which has been made in nutritive studies of these crops during the past years. Some years ago, while traveling through a small town in northern Iowa, I noted a sign at the edge of town which stated "Speed Limit 25 miles per hour - $3.00 per mile an hour thereafter. Pick the speed you can afford." For years and years, agricultural processors would have picked the lowest speed. Today we are moving faster and paying more to properly control all aspects of harvesting and processing agricultural crops. In general, the dehydrator is concerned with three phases of quality control--the care and development of the plant prior to harvesting, the harvesting and processing methods employed by the dehydrator, and finally the methods by which the processed materials are stored and reprocessed for final usage.

As a processor of dehydrated alfalfa, our control of growth and development of the crop depends to a great extent on the cooperation of the individual growers with the various agencies supplying them with up-to-date information on such methods. In this area we are particularly dependent on the knowledge and information furnished by the Department of Agriculture and their agencies, various county agents, as well as the many state universities and agricultural information media. The excellent work which has been done by these agencies in recent years to provide information for improving soil fertility and conservation, as well as encouraging the use of registered seed selection in proven varietal strains, is most beneficial to the dehydrators as well as the growers. Other projects such as weed control methods, deterring factors that may be provided to insect predators, both of which have included extensive field tests, with careful analysis of results and statistical and economic information to justify the grower taking positive steps to institute proven methods of control, are of inestimable value. The alfalfa aphid infestation suffered by the industry in recent years is a revealing case in point. Various government and educational agencies throughout the country combined in an all-out effort to alleviate the plague and, speaking for our own organization and as a representative of the dehydrating industry, I would like to acknowledge to these hard working, responsible people, the debt we all have for preventing what might have been real catastrophe in the alfalfa industry. The war is not won but sufficient battles have been taken with new and improved chemicals, laboratory-developed predators such as molds, wasps, beetles, etc., together with fantastic speed in developing new varieties of alfalfa sufficiently resistant to withstand the depredation of the alfalfa aphid.

Field renovation and the hitherto unheard-of process of applying fertilizer direct to forage crops are further evidence of the progress afforded growers and processors alike by the continuing good work of the government agricultural experimenting agencies and their representatives. Once the plant has been properly selected, nurtured, protected, and grown to maturity, the harvesting methods which are incumbent on the processor begin to play an important part in maintaining and preserving the value which has been bred and developed into the plant by the grower with his modern agricultural methods.

The efficiency and quality preservation accomplished in the field depends on when and how the hay is cut--many people refer to hay as best harvested when in the 1/10 bloom stage. For individual grower--cutting his own crop--this time reference has more significance than to the dehydrator. This is mentioned since after first cutting the dehydration mill operator must cut his fields in rotation. Therefore, his guide must necessarily be the time between the cuttings rather than selecting the field by its indicated maturity in bloom. By trial and error we have found that year-in and year-out hay that is cut between 28 and 32 days after the previous cutting has consistently yielded first quality with relation to quantity that can be achieved on this rotation basis. Interestingly enough, the amount of bloom varies considerably within this time cycle. This is due mainly to the moisture and temperature but is also dependent on the area being cut and which cutting is being harvested. Hay less than 33 days old can almost always be depended on to yield 17% or better protein and 135,000 or better carotene. Kansas fields cut on this basis usually amount to a little less than one ton per acre cutting (providing severe drought or insect depredation are not factors). Colorado fields approximate a ton to the acre cutting and Nebraska yields are between 1 1/8 and 1 1/4 tons. These figures represent averages for all cuttings and for three or more years' production.

One day last week I read a news release by a Japanese firm announcing the successful design of a radio-controlled motorized golf cart. The next logical step, of course, will be a machine that makes all of the shots while you sit in the clubhouse playing gin rummy.. Alfalfa harvesting machines have gradually improved over the years but they are still not a push-button operation. Several factors of design vitally affect the quality of the product. Weight of the machine or machines is one of the more vital considerations. Many acres of alfalfa are lost every year because of heavy harvesting equipment, particularly when they are used on muddy or wet fields. Since the machines are more complicated and power is increasing each year, the only answer seems to be better flotation with large airplane type tires so the weight may be distributed over a greater area and provide less damaging loads per square inch. An interesting example of the reverse of this principle has been pinpointed by the engineers for a floor covering manufacturer. When their floors were noticed to be deteriorating at a very accelerated rate in a Washington, D. C., building recently, they investigated and found that many of the women employees were wearing the new, very narrow high heels now in vogue and the pressure exerted by the ladies in these heels during normal walking was several hundred pounds to the square inch. Now I am not an engineer but I could have told this company about these extreme pressures sometime ago--have you ever tried dancing with your wife in a roomful of teenagers attired in high heels?

Other design factors such as mobility and productivity must be considered in building an economically sound field harvester. Mowing and chopping features, however, are of prime concern from all standpoints. Bert & Wetta currently distribute what appears to be the best answer for the mowing problem. It is an assembly utilizing two counter-driven sickle bars. This device gives better cutting action with half the speed and movement utilized on the older type mowers and enables excellent field cleanup on each cutting. If Joe Wetta is here, I want two more of these sickles for free after this plug! Fine chopping, which we feel is the real key to quality production, is done by the cutter head. There seem to be about as many types of cutter heads as there are varieties of field machines. All types have various advantages and disadvantages involving horsepower, ease of sharpening, resistance to foreign objects, accessibility, etc. The primary value, regardless of these, however, is the fineness and cleanness of the chop. The shorter the stem and the sharper the cut of

separation, the better the final product will be. The main problem of dehydration (if you will permit another homespun analogy) is somewhat like a mother trying to dry the baby's diapers and his formula bottles, all thrown in the family clothes dryer for the same length of time. The leaf, like the diaper, exposes a large flat area to the heated air stream in the drum, which enables it to transfer the unwanted moisture quickly. The stem is like the baby's bottle--it is heavy and has the moisture entrapped where the air stream can't get at it as well, so the drying process is slower and less efficient. It is true that the leaf, once dry, moves more rapidly through the drum and we come out with about the same moisture, providing the drum is of properly designed length and diameter, is being rotated at the right speed, the burner is correctly adjusted, and the fan is moving enough air in relation to all the other factors. Obviously, this string of circumstances would be nebulous enough if we cut all hay at the same time of the day and moisture content were exactly the same, which is obviously not the case. The best answer then would seem to be to make the stem as much like the leaf, from a moisture release standpoint, as possible. Only in this way can we eliminate wet stems, burned or scalded leaves, and get a uniform product, regardless of all other variables.

Strange as it may seem, temperatures at the apertures of the drum apparently have little effect on quality. We have analyzed the product from the same field with variations from 1100° F. to 1500° F., with no appreciable differences in protein and carotene content. Exit or drum end temperatures are a very different story. Variations of as little as 25° F. can make considerable difference in quality and moisture content. With some drum designs, alfalfa actually gains moisture the last 8 or 10 feet when the end temperature is too low. Exit temperatures that are too high will burn leaves and cause quality robbing separation, which is apparently quite damaging.

After the material is dry and has passed through the main cooling collector, grinding must be properly accomplished if we are to avoid "dropping the omelet". Sharp knives and sharp screens with extremely high tip velocities in the hammermill seems to do the best job. If the job is done quickly enough to avoid high temperatures, we maintain quality with little or no loss. Caution at this point seems to be the absolute ascertainment of grinding capacity. Any factor which might interfere with grinding at high speed will tumble quality at an alarming rate.

Most dehydrators pellet the product at this stage of the game. Pelleting equipment, like harvesting equipment, is of many makes and varieties. In all pellet machines we know that pressures and temperatures skyrocket. No one knows for sure but estimates of 20,000 psi and 1200° F. have been estimated. We do know that pelleting as a process seems to have little or no effect on quality. Temperatures and pressures notwithstanding, the action is so rapid that there are no apparent lasting effects from what would appear to be damaging methods. By the same token, pelleted material has no better quality retention factors by virtue of its being pelleted. The only matter in this regard of significance seems to be that the inert gas atmosphere used in storing the product will pass more readily through pelleted material, thereby eliminating damaging oxygen far more readily than other forms of stored dehydrated material. One thing is certain, quality storage under inert gas can be accomplished only if the pellets are relatively free of fines and the gas atmosphere of the storage container is constantly and reliably checked for oxygen. Under properly designed and maintained environment, carotene levels can be maintained indefinitely at 98 and 99% of the input values.

One disturbing phenomenon whichoccurs under gas storage is difficult, if not impossible, to control. This is moisture transfer. Most inert gas is dehumidified before introduction. However, it warms rapidly in contact with the stored pellets. As the gas goes up in temperature, it goes up in moisture carrying capacity and content. It actually absorbs the moisture from the stored pellets and then dumps it out by condensation when contact with the outer walls of the container reduces its temperature and moisture carrying ability. The resulting condensation collects in the pellets nearest the walls in relatively large amounts, causing soggy masses of pellets which will freeze into rock hard lumps if temperatures are low enough. These lumps clog or damage handling equipment and interfere with blending procedures. Expensive insulation methods are obviously one answer, but the problem is far from solution, particularly in steel storage tanks.

Another unusual characteristic which has been observed since the advent of gas storage is the quality retention variation between material that has been grown and procured under normal environment and alfalfa that has undergone damage of some kind during the growing stage. Although hay which has been frozen or hailed will show no great damage by present laboratory methods at most stages, it will lose carotene after gas storage at a greater rate than otherwise undamaged material.

I have attempted to present to you some of the problems which are inherent in the dehydration, processing and storage of alfalfa. Through the years much progress has been made and still much additional progress can and, I know, will be made, not only in the processing and preservation of forage crops but in the use, from a proper nutritive standpoint. The importance of properly applied research, from both the processing and use standpoint, can never be underestimated. I am sure that closer cooperation between research groups, agricultural processors, and the ultimate users of our products will benefit us all.

FARM RESEARCH ON ALFALFA

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Agronomists share with the alfalfa growers, processors of alfalfa products, and stockmen the importance of efficient production and a high-quality product in today's economy. Adapted varieties and sound cultural practices which assure stable production and high quality feed at a reasonable cost are the first steps of a path leading to a competitive alfalfa product. I have taken the liberty of confining most of my remarks to recent advances in genetic and agronomic research on alfalfa which bear immediately on the role of alfalfa in a competitive feed program. While concentrating on the applied aspects, however, I do not wish to overlook the contributions from the more fundamental studies. I have reserved my concluding remarks for quality in alfalfa, where an intensification of effort would be expected to bear fruitful results.

A dramatic expansion of the alfalfa acreage in the United States is shown by hay and seed statistics for the past 40 years (table 1). Hay acreages in the United States increased two and one-half million during each of two decades following 1920. There was an increase of about four and one-half million between 1940 and 1950, and nine and one-half million since 1950. New varieties, adequate quantities of high-quality seed, and increased interest in alfalfa appeared to be the principal factors associated with the tremendous increase in acreage during the past decade. The bulk of the increase occurred in the North Central States, although there was a positive increase in all regions of the United States. In 1958, about 65 percent of the alfalfa in the United States was grown in the region defined by the census takers as the North Central States.

A marked shift in the area of alfalfa seed production also occurred during the past decade, the shift occurring principally from the Midwestern to the Southwestern States. In 1958 an estimated 43 percent of the United States alfalfa seed crop was grown in California alone. Ample quantities of high-quality seed of the improved varieties are now generally available.

Alfalfa workers have held regional conferences in the odd-numbered years to discuss problems and progress in genetics, breeding, pathology, entomology, physiology, ecology and seed production. A continental conference, which includes Canadian workers, is held in the years alternating with the regional conferences. These conferences have become a stimulating and an effective medium for exchanging information, ideas and materials, and for coordinating tests of promising strains. Teamwork among specialists in the various subject-matter fields has become increasingly apparent in the field of alfalfa improvement.

Current work on the genetics of alfalfa includes studies on the inheritance of agronomic characters, and on disease and insect resistance. Considerable attention is being given to the development of breeding procedures which will result in the maximum expression of hybrid vigor in a commercial synthetic or hybrid. Other information bearing on the development of efficient breeding procedures is being obtained from studies on the nature of polyploidy, the self-incompatibility mechanism, identification of linkage groups, and cytogenetic behavior.

The onslaught of the spotted alfalfa aphid diverted many breeding programs to a search for resistance to this pest. Soon after the discovery of this aphid, resistance was found in the variety Lahontan. Three varieties have since been released with resistance to the spotted alfalfa aphid. Moapa was developed by the U. S. Department of Agriculture and the Nevada Agricultural Experiment Station and released jointly by these two agencies and the Agricultural Experiment Stations of Arizona and California in 1957. Moapa is a nonhardy variety similar to African and adapted to the Deep South and Southwest. Zia was developed by the New Mexico Agricultural Experiment Station, and named in 1958. It appears to be adapted to New Mexico and possibly other areas. Cody is the most recent addition to the list of new varieties resistant to the spotted alfalfa aphid. It was developed by the Kansas Agricultural Experiment Station and the U. S. Department of Agriculture, and released in 1959. It has a high degree of resistance of the antibiosis and tolerance types. In other respects, Cody is very similar to the variety Buffalo. It is generally agreed that every new variety which is to be released for the midwestern, southwestern, and southern parts of the United States should have resistance to the spotted alfalfa aphid.

Speaking of new varieties, Teton, a promising pasture-type of alfalfa for the Northern Great Plains area, was released in 1958 by the South Dakota Agricultural Experiment Station. It has a low, wide crown with aggressive rhizome development. The new variety is resistant to bacterial wilt and is very winter hardy. It appears to offer greater promise for grazing than for hay.

Efforts are being renewed to develop varieties resistant to the pea aphid. Resistant plants have been isolated, but additional work is necessary to combine resistance with other desirable characters. Genes for resistance to the spotted alfalfa aphid do not appear to confer resistance to the pea aphid.

Considerable work is in progress on the inheritance of the creeping-rooted character and the development of adapted varieties bred for this trait. This type of spreading results from the production of adventitious stem buds at enlarged points along the length of lateral underground roots.

Recently, biotypes or races of the spotted alfalfa aphid (8) and the bacterial wilt organism (4) were discovered. The presence of biotypes which react differentially on a given set of alfalfa clones causes additional complexity in breeding for resistance. Such information, however, is mandatory for a realistic attack on disease and insect problems. In the North Central States, the different species of micro-organisms involved in the black stem disease complex of alfalfa have been identified (3, 9 and others). Progress has been made in breeding for resistance to some of them. Experimental strains have been developed in the Northeastern and Eastern States with higher levels of disease resistance than are available in present varieties.

The development of a high seed- and forage-yielding synthetic in Utah is encouraging from the standpoint of producing seed at lower cost. Growers, of course, prefer to produce seed of varieties with a high seed-producing potential. The Utah synthetic has not been released.

In Denmark, studies by Nielsen, et al. (7), on the effects of frequency and time of cutting on top and root growth and on organic root reserves in alfalfa were in general agreement with earlier studies by Graber, et al. (5), and Grandfield (6).

There is some evidence that varieties may respond differently to additional stress imposed by more frequent cutting. At Madison, Wisconsin, studies by Dr. Dale Smith (oral communication) indicate that the superiority of Vernal over other commonly used varieties was greater when harvested three times during the season than when the varieties were harvested twice. The criteria for comparison were yield and persistence.

FACTORS AFFECTING QUALITY

Diseases and insects. Many factors appear to affect the quality of alfalfa hay. Depradations from a host of diseases and insect pests are recognized as common causes for low quality. Their effects on quality are difficult to study and only a few data are available. In Iowa, Brigham (1) determined the chemical composition of leaves infected with the fungus Cercospora medicaginis. Leaves which were one-eighth diseases contained only 60 percent as much crude protein and 75 percent as much ether extract as disease-free leaves. Crude fiber increased 15 percent, suggesting a decrease in digestibility. The inference was made that the fungus was able to draw on nutrients in the area surrounding the lesion without actually being established in that region.

In Wisconsin, Smith and Medler (10) used insecticides to control leafhoppers on Vernal and Narragansett alfalfa to study the effects of leafhoppers on yield and chemical composition at two levels of soil fertility. The insects reduced the hay yields of Vernal 21 percent and Narragansett 28 percent with high soil fertility, and 36 percent and 48 percent, respectively, with low fertility. There was only an 11 percent difference in hay yields with both varieties due to soil fertility alone. Reductions in the yield of protein, ash, Ca, and P were generally greater than reductions in hay yields. The average reduction of these four constituents amounted to 33 percent for Vernal and 38 percent for Narragansett under high soil fertility, and 41 percent and 55 percent, respectively, under low soil fertility.

Spotted alfalfa aphids are known to reduce the quality as well as the quantity of forage. Leaves are lost, carotene and protein contents are greatly reduced, and fungi grow readily on the honeydew given off by the aphids.

In Georgia, Burton (2) found that anthracnose affected the yield of Sudan grass very lightly; yet, the disease caused a 9 percent reduction in protein and fat content.

The preliminary evidence indicates that losses in quality from diseases and insects is much greater than one would expect on the basis of leaf shattering and the appearance of the forage alone. While considerable advance has been made in improving the quality of alfalfa through breeding, increases in disease and insect resistance continues to be a very fruitful area of research. Increased resistance to any one of about 20 diseases or insects no doubt would improve performance and quality.

Biochemical constituents affecting quality. The staff of the Laboratory here at Albany and others have made notable progress in the identification and determination of biochemical constituents in alfalfa which affect its value as feed. Notable advances have also been made in determining their physiological effects on farm stock. Different lots of alfalfa which have been studied chemically differed tremendously in contents of saponins, estrogenic-like substances, and vitamins. From the standpoint of improving the feeding value of alfalfa, increasing or lowering the quantity of specific chemical constituents through breeding is indeed challenging.

One of the first needs, however, is for more information on the over-all causes of variation. The Alfalfa Section, in cooperation with State Experiment Stations and the Western Regional Research Laboratory, is now in the process of collecting forage samples of alfalfa varieties at diverse locations in the United States. Data are being obtained in such a manner as to obtain information on the effects of variety, cutting, location, season, stage of maturity, disease incidence, and specific elements of the environment. Statistical procedures are available for the design of experiments and the analysis of the data which can be used with confidence to estimate the proportion of the total variation attributable to the respective sources of variation. Information of this sort is needed in the development of effective research programs in breeding or management of alfalfa designed to alter chemical composition. Again, close cooperation between agronomists, biochemists, nutritionists, pathologists and entomologists is extremely important for greatest effectiveness. Even with good cooperation prevailing, however, limitations on the number of chemical determinations which can be made is serious. Chemical analyses are expensive. As a matter of comparison, it is usually possible to score many plants or strains for agronomic or disease characteristics at a cost of a single chemical determination.

I have attempted to review some of the recent highlights in agronomic and genetic research on alfalfa.

Table 1. Acreages of Alfalfa Hay Harvested in the United States During the Period 1920-1958[1/]

States	1,000 Acres					
	1920	1930	1940	1950	1956	1958[2/]
North Atlantic	191	365	794	1,312	2,032	2,407
North Central	3,951	5,753	7,639	12,181	19,458	19,419
South Atlantic	52	85	152	382	611	677
South Central	555	433	815	1,164	1,270	1,241
Western	4,266	4,973	4,623	4,862	6,031	6,057
United States	9,015	11,609	14,023	19,901	29,402	29,801

1/ From "Agricultural Statistics," U. S. Department of Agriculture. Alfalfa hay until 1949, when alfalfa hay mixtures were also included.

2/ Preliminary.

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ENGINEERING ASPECTS OF HAY WAFERING AND PELLETING

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Field curing and harvesting procedure is important to hay pelleting. This is true whether the hay is to be processed in a remote stationary plant or by a field pelleter. Fortunately, good quality, leafy hay is easier to process than low quality hay. Since good quality hay also makes the best feed, pelleting should be one more reason for good hay making practice.

Proper attention to field operations, such as mowing, conditioning, raking, baling, and chopping can provide a leafy product of uniform and safe moisture content. Such hay can be ground with a minimum of difficulty and provides a uniform meal that makes a good pellet at maximum machine capacity. On the other hand, improper operation of field equipment can result in irregular windrows, spotty curing and excessive loss of leaf, all of which detract from the pelletability of the hay.

Materials handling starts in the hay field. The usual method of handling hay to the pellet mill is in baled form. Bales are stockpiled, then moved to the pellet mill from the stockpile, usually on pallets.

Chopped hay is gaining in popularity as a means of handling hay to the pellet mill. During the haying season, chopped hay is hauled directly from the field to the pellet mill grinder. For off-season use hay may be stored either in chopped or baled form. Chopped hay must be thoroughly cured in the field to facilitate immediate grinding.

Field pelleting, as yet in the development stage, will probably replace field baling and chopping to some extent. Increased density and bulk handling will make field pellets better for long hauls and for storage. For short hauls, chopped hay may still be the most economical method of handling hay for direct feeding or for processing into small pellets. Cost of field pelleting, as yet undetermined, will be the deciding factor.

Field vs. Stationary Plant Pelleting. Stationary plant pelleting, currently increasing in use, is not likely to be replaced by field pelleting but should be supplemented by it. For all-roughage feeds, field pellets may be preferred to the ground-hay pellets produced in a stationary plant for certain animals. However, where mixed-ration pellets are desired, feed preparation and formulation are obviously fixed plant operations. If field pelleting can compete economically with baled or chopped hay, much of the production of field pelleters may be reprocessed into ground-feed pellets.

Field pelleting is somewhat more dependent on hay and weather conditions than stationary plant operation. Part of the problem in developing field pelleters is in producing a machine that will operate under a wide variety of field conditions. Most field pelleters will be limited to one predetermined size and shape of pellet.

Pellet Handling. Finely ground hay pellets have optimum bulk handling characteristics. They are free-flowing, can be easily separated from fines, and have maximum density for direct feeding to animals. They may be handled either mechanically or pneumatically, although screw conveying usually results in excessive fines.

Field pellets, because of their shape and larger size, do not flow well. However, through the use of mechanical aids, they can be readily handled in bulk. Field pellets vary from 1 1/2 to 2 times the density of baled hay.

Current Status of Field Pelleting. Field hay pelleters are still in the experimental stage. Several machinery companies have machines under development. Indications are that it will be at least 1 1/2 to 2 years before field pelleters will be available commercially. Several types are being tested. Current models have estimated capacities of 3 to 5 tons per hour. Estimates on the first cost of these machines vary, but it is expected that field pelleting will cost as much or more than field baling.

Although some machines have occasionally worked successfully in hay up to 30 or 35% m.c., optimum moisture generally ranges from 15 to 25%. Field machines take hay directly from the windrow, delivering pellets into a trailer. Varying field conditions may result in a wide variation in pellet density and quality.

REVIEW OF THE ADA RESEARCH PROGRAM

Joseph Chrisman

American Dehydrators Association
Kansas City, Missouri

The American Dehydrators Association owes much to the people in the United States Department of Agriculture, and particularly to the people in the Western Regional Research Laboratory, both past and present.

Shortly after our ADA sponsored research program took shape in 1949 we drew upon the bank of forage crop research knowledge here at Albany and we have had a checking account on this bank ever since. I am afraid our withdrawals have exceeded our deposits.

Back in 1951, Lloyd Larson, then secretary of ADA; Dayton Maclay, then head of the Biochemical Division here and also chairman of our relatively new Alfalfa Research Council; and Dr. M. J. Copley, who has just welcomed us to this beautiful spot on the shores of San Francisco Bay, conceived the idea of holding a conference of forage processors, growers, users and research workers, for an interchange of knowledge and discussion of current operating problems and research progress.

At that first meeting in April, 1951, there were some 70 research and industry people in attendance from 14 States. Jerry Field struck the keynote of the feeling within our industry then, and I believe it is as true now as it was then. He said, "No industry can survive on false premises. The alfalfa industry can maintain and expand market outlets only so long as it has facts and truth with which to work."

In that 1951-52 year our industry produced and marketed 850,000 tons of dehydrated forage crops. In the year ended, on April 30, 1959, the records show a production of 1,100,000 tons, an increase of 30 percent and a disappearance or usage of 1,156,000 tons. The industry has grown by about one-third in the eight years following that first conference.

By continuing and expanding our research and by making good and proper use of the research findings, I am confident this growth of market will continue and perhaps at an accelerated pace.

In the eight years we have enjoyed the benefits of many new and better varieties of alfalfa. We have learned how to improve stands through better fertilizing practices. Destructive insects are being held under control to a much greater degree. We have learned much about the feeding of the product to all species of animals.

Back of all of these improvements and very largely responsible for them, lie years of research, much of it done in the Field Crops Laboratory of this fine institution we are visiting today.

We in ADA are proud of the fact that we, too, have contributed to building a reserve of knowledge through our own research program. Since 1949 we have contributed to various experiment stations for research in

Dairy.....	$ 23,050
Poultry.....	44,812
Beef.....	24,350
Sheep.....	21,368
Swine.....	8,000
Chemical.....	19,367
Antioxidants.....	9,500
Economic.....	5,000
Agronomic.....	34,190
	$189,637

We have made some further contribution to the dissemination of knowledge by printing and distributing, free of charge, over 900 letter-size pages of Alfalfa Abstracts. These abstracts cover production, harvesting, composition, and uses, and are by no means limited to the dehydrated alfalfa field.

Currently we have projects toward which we lend financial support at the University of California at Davis entitled "Methods of Feeding Dehydrated Alfalfa Pellets as a Supplement for Range Sheep." Dr. J. H. Meyer, whom you will hear later on this program, is the project leader there. This will be the fifth year of our participation in research at Davis under Dr. Meyer.

At Cornell University, Dr. Fred Hill is concluding work on "Studies of Energy Value of Alfalfa Products for Poultry" of three years' duration.

Michigan State University is entering its third year of work concerning "The Nutritive Value of Artificially Dehydrated Alfalfa Pellets for Dairy Cattle." This project was under the leadership of Dr. C. A. Lassiter, who has recently been named head of the Dairy Department.

A new project was instituted this year at the University of Florida under Dr. T. J. Cunha. The title of this one if "The Effect of Protein Level and Dehydrated Alfalfa Meal on Reproductive Performance of Young Lactating Beef Heifers on a Grass Hay Wintering Rations."

Another new one this year is placed at South Dakota State College under Dr. R. C. Wahlstrom entitled "The Value of Artificially Dehydrated Alfalfa in Dry Lot Rations for Swine During Growth, Gestation and Lactation."

Wisconsin Alumni Research Foundation is making further investigations on the availability of the Vitamin E in dehydrated alfalfa under our sponsorship and financing.

Two years' work at the University of Nebraska, under Dr. J. K. Matsushima, gave good evidence of the presence of estrogenic-like activity in alfalfa when fed to fattening beef cattle. The two years' work gave such closely corresponding data that a third year's grant was not requested. However, the work at Nebraska is continuing.

Workers at this Western Regional Research Laboratory had spotted two 5-ton lots of dehydrated alfalfa in the Middle West Region, one of which, by mouse assay, proved very high in estrogenic activity and the other extremely low. This

presented an excellent opportunity to make further studies, not only at Nebraska but elsewhere and through joint efforts of the Field Crops Laboratory here at Albany and ADA we placed three tons of each lot at Nebraska, one ton of each at Oregon State College for sheep feeding under Dr. Oldfield, and one ton of each at Beltsville for dairy cattle feeding under Dr. Sykes. The Association's contribution was in purchase of the alfalfa and payment of the freight. Incidentally, the freight amounted to more than the cost of the alfalfa in several instances.

We feel that there is a great need for further research in forage crop composition and utilization. Much of the work that needs doing is in the field of fundamental research rather than so-called practical research. We feel that the Field Crops Laboratory of this institution is most ideally suited and equipped to pursue these studies.

Recently policy statements of the Food and Drug Administration with regard to the new food additives law and the Delaney Amendment haveplaced the synthetic estrogenic substances in a sort of twilight zone. Nobody knows just now what their eventual status may be. Without, at this time, taking any pro or con stand on the controversial issue, we do have one firm conviction: That studies should be speeded up on the estrogenic activity in some of our green forage crops. Why are some lots high and others low? What are the effects of stage of growth, soil fertility, age of stand, crop or cutting, season, variety, etc? What is a good accurate method of quantitative analysis? What, if any, losses take place in processing and storage and how can these losses be avoided?

With these things in mind, the Association in the person of its executive vice president called upon the top officials of Agricultural Research Service in Washington a few weeks ago to plead the cause of accelerated research along these lines.

The Western Lab has done much fine work already in studying this phase of alfalfa composition, even to the extent of isolation of coumestrol. To do more work than is presently being done would require more men on the particular job and that, of course, means more money. We believe that this is a top priority job under existing circumstances, not only for the dehydrating industry, but for the feed industry and the feeders of cattle and sheep throughout the land. Better than 50% of the total feed intake of our livestock population is in our forage crops. Let's know more about these crops.

Some exceptionally fine work has been done at Purdue University by Dr. Fred Andrews and his associates in studying the estrogens in forages with relation to varieties and strains and also with respect to stage of growth and crop and under various methods of ensiling.

Enough results have been published to point up the importance of this line of study and, so it seems to us, to make it imperative that the subject be pursued with even greater vigor in the immediate future.

The Association expects to continue its contributions to the research efforts and when possible to expand them. We stand ready to assist in any way we can, the researches under way in Agricultural Research Service facilities and the various Experiment Stations over the country. We can never set limits on learning all the truth about so important a national and international crop as the forage crop.

USE OF PELLETED AND OTHER FORMS OF ALFALFA IN RUMINANT FEEDING

J. H. Meyer

University of California
Davis, California

Improvement in harvesting, transporting, storing and feeding of hay not only influences the economics of hay handling but it may also influence the feeding value of the hay. Pelleting is such a procedure. In general, particularly when the largest proportion of the ration is hay, increased rate of gain resulted when the ration was pelleted. When larger gains were observed on pelleted feeds, an increased feed consumption was generally found. Results from one experiment (table 1) demonstrate increased gain can be obtained when high-quality, dehydrated hay or field-cured hay is ground through a fine screen and pelleted. Increased feed intake and efficiency of feed utilization were noted. These animals consumed a greater quantity of the pelleted hay, and thus a larger percentage of the feed was used for gain and less for maintenance. Hence, this is the primary reason for an increased efficiency of feed utilization.

Table 1. Results with Sheep Comparing Chopped and Pelleted Finely Ground Hay (56 Days)

	Dehydrated		Field-cured	
	Pelleted	Chopped	Pelleted	Chopped
No. of lambs	20	20	20	20
Daily gain, lb.	0.42	0.30	0.33	0.16
Daily feed, lb.	3.8	3.2	3.5	2.3
Feed/100 lb. gain, lb.	900	1070	1070	1480

Further research was conducted (table 2) with the equalized feeding technique to investigate whether animals fed an equal quantity of chopped and pelleted hay would make the same weight gain. These results indicate no difference in the rate of gain of sheep fed the same quantity of chopped hay or pelleted hay. This confirms previous work done at the University of Illinois.

Table 2. Results from the Equalized Feeding of Chopped and Pelleted, Finely Ground Hay (56 Days)

	Maintenance		Rapid Growth		
	Chopped	Pelleted	Chopped	Pelleted	Pelleted
Feed consumed, lb.	1.66	1.67	2.48	2.54	3.25
Daily gain, lb.	-0.02	-0.01	0.27	0.26	0.40

Further investigation was made of the energy content of alfalfa hay when utilized as chopped or pelleted, finely ground hay. There was no significant difference between the energy content of chopped or pelleted alfalfa hay (table 3).

This is in contrast, with the exception of net energy, to the results reported by Blaxter and Graham (Jour. Agric. Sci. 47: 207, 1956). They reported a lower TDN, digestible and metabolizable energy in pelleted, ground grass hay.

Table 3. Energy Content of Alfalfa

	Chopped	Pelleted
TDN, %	58.7	59.5
Gross energy[a]	205	202
Digestible energy[a]	124	127
Metabolizable energy[a]	102	104
Net energy[a]	63	65

[a]Megacalories per 100 lb. dry matter

In one experiment we killed groups of sheep at prescribed intervals after feeding and made studies of the digestive tract contents when fed either chopped or pelleted hay. The total and dry matter content of the reticulo-rumen was less for the pelleted hay-fed sheep than those fed chopped hay. The smaller content of the reticulo-rumen of the pelleted hay-fed animals was mainly due to lower holocellulose. Therefore, this seems to indicate a faster passage of feed from the reticulo-rumen of the pelleted hay-fed sheep rather than any differences further along in the tract.

The studies of the fatty acid production by rumen content in the Warburg confirmed that a faster rate of digestion occurs when the hay is ground and pelleted. A much greater fatty acid production from the rumen contents occurred at 1 1/2 and 4 hours after feeding for the pelleted hay-fed animals as compared to chopped hay-fed animals. In addition, the lignin-holocellulose ratios of the rumen contents indicated a faster breakdown of holocellulose by the rumen micro-organisms of the sheep fed pelleted hay.

It was postulated that the increased feed intake resulting from grinding and pelleting hay is the direct result of a faster rate of digestion in the reticulo-rumen. This accelerated digestion allows a faster passage of feed through the digestive tract. The finer hay particle size, more logically, might be the reason for this. Therefore, a further experiment was designed to study this hypothesis. In this experiment, one-half of the hay was ground through a 1/16-inch screen, while the other half was coarsely chopped (1-inch screen). Four identical treatments were designed for each batch of hay--the finely ground and the coarsely chopped hay. The treatments were: (1) Fed as finely ground or chopped hay; (2) fed as a pellet; (3) fed as ground or chopped hay mixed with water; and (4) fed as the reground pellet mixed with water. An inspection of these data (table 4) provides some evidence for the suggestion that fine grinding may be a major factor for the increased feed consumption of the pellet, and that pelleting serves to put a dusty feed in a more palatable form. The evidence is: (1) The sheep fed the finely ground material had the largest feed intake; (2) adding water to the finely ground material resulted in feed consumption practically as great as from pelleting the material; and (3) feed consumption did not increase as markedly when coarse material was pelleted as when fine material was pelleted.

Table 4. Response of Sheep to Pelleted, Finely Ground Hay and Pelleted, Coarsely Chopped Hay (Wafer)

	Ground	Pellet	Ground plus water	Reground pellet plus water	Mean
Finely ground:					
Daily gain, lb.	0.20	0.38	0.31	0.35	0.31
Daily feed, lb.	2.03	3.59	3.17	3.12	2.98
Chopped:					
Daily gain, lb.	0.24	0.30	0.19	0.30	0.26
Daily feed, lb.	2.80	2.86	2.54	2.98	2.80

REVIEW OF GRASS JUICE INVESTIGATIONS

G. O. Kohler

Wheat milling is a typical process used to upgrade an agricultural crop to increase its value. Wheat flour is used by human beings in bread and other baked goods. Wheat bran and other byproducts are used as animal feedstuffs.

The idea of developing a comparable process for forage crops is probably very old, but serious efforts in this direction are relatively new. There are several European reports relating to separation of high and low fiber fractions from forages dating back to the early 1930's. In this country some of the earliest recorded researches pointed in this direction date back to the days of the first World War.

Early interest in both Europe and this country was focused on production of a vitamin and protein concentrate suitable for human diets. Good forages will produce several times more protein per acre than soybeans, for example. However, it was found that neither economics or technology of today will permit use of forages to produce high protein concentrates to compete with corn and soy protein if no other high value byproducts can be formed.

Current interest in a "milling" process for forages is based upon several considerations. First, fresh forages and concentrated forage juice contain important labile unidentified growth factors which are largely destroyed by conventional dehydration and by hay-making. Second, the use of whole, dehydrated forages in poultry and swine feeding is limited because of their high content of celluloses and lignin, or stated another way because of their low-energy content. Third, dehydrated alfalfa, the forage best adapted for large-scale production, contains growth inhibitors for poultry and swine which might be eliminated by a proper fractionation.

Mechanical fractionation of dehydrated alfalfa can reduce the fiber problem but cannot bring back a destroyed growth factor or eliminate the growth inhibitor.

Let me turn then to the biological basis for a juicing process. How do we know that forage juice contains an unidentified growth factor? Where chicks were fed a ration (Chart 1) complete in protein, minerals, and all known vitamins, they grew at a reasonably good rate. Adding 3% of forage juice concentrated caused an increase in growth rate of 5 to 15%. Adding higher levels of all known vitamins, including inositol, choline ascorbic acid and all of the B vitamins did not have any effect on growth rate.

Further results have shown that the growth factor is destroyed to a large extent during dehydration (Charts 2 and 3). Thus 1% of dehydrated alfalfa can be used in the basal ration as a source of carotene. Pretreatment of the forage before dehydration with alkali or with acid or sulfite had little if any stabilizing effect on the growth factor. Type of forage had no effect on potency, alfalfa juice being as good as cereal grass juice. Fish solubles contain a different unidentified growth factor than forage juice. When fed individually and in combination, fish solubles and forage juice give mutually complimentary growth effects. The lability of the forage juice factor to dehydration has been demonstrated either in the presence or absence of fish solubles.

In all of the chick work, a major problem has been the assay procedure. Occasionally the growth response would be lost and much time and energy wasted. One of the factors affecting response was season of the year. In an experiment over an 8-month period (Chart 4), in which assays were set up every 2 or 3 weeks, it was found that the response was lost entirely in May and June. Since commercial chicks were used, this loss is attributed to carryover of the growth factor from hens on lush range in April and May.

During the period when these experiments were in progress, similar work was being done at Cornell University by Drs. Norris and Scott and their associates under a Cerophyl Laboratories Grant-in-Aid. A great deal of work on the chick assay procedure was done at Cornell. The most successful ration used was one based on fishmeal as a source of protein. (Chart 5).

Other research on chicks has been reported from many experiment stations. As an example, work was done at the South Dakota Experiment Station (Chart 6) in which forage juice, when added to a basal ration containing aureomycin, gave a good growth response over the controls.

Turkey poults have consistently given better assay results than chicks. Work at Cornell has shown that the forage factor deficiency is usually the first limiting factor for growth of poults. The turkey poult studies showed that dehydration destroys most of the growth factor (Chart 7). Also, it was shown that juice from grasses was equivalent in activity to alfalfa juice. It was shown further that antibiotic had a sparing effect on the unidentified forage juice factor (Charts 8 and 9). In rations fed antibiotic, forage juice produced a growth rate increase of 5 to 10%. In the absence of antibiotic, the forage juice produced increases of 25 to 35%.

I should next like to describe some very important experiments done at Cornell which show that the GF should be fed to laying turkey hens in order to obtain optimal results in their offspring (Chart 10). Two groups of hens were fed a good practical ration; one receiving 5% forage juice while the other did not. Poults from each group were divided into subgroups which were fed the basal poult ration alone and with added forage juice concentrate, respectively. Mortality was high and poult growth was poor where neither poults nor dams received forage juice. Feeding juice to the dams improved poult growth and mortality regardless of whether juice was fed to the poults. Feeding juice to the poults improved growth and reduced mortality regardless of the dams' diet. But in order to obtain optimum results, juice had to be fed to both. Here results are superior to those obtained on a commercial ration.

Similar experiments demonstrating carryover of GF from turkey hens to their poults have been carried out by Dr. Slinger at Ontario Agricultural College and by Dr. Wilcox at the South Dakota Experiment Station.

So much for the work on GF in poultry. I should like to emphasize that the results I have discussed are strictly representative and constitute only a small fraction of the total work done. The great bulk of the results has been positive. Several workers have reported little or no response under their conditions. In most of these cases the tests were run as incidentals to other research and no sustained effort was made to adjust conditions so as to obtain a response. Indeed in the single experiment we have run here at the Western Regional Laboratory we did not get a response in our chicks. Whether this is due to carryover from the hens, to strain of chicks, inactive juice or other variables is not known.

Before leaving the biological background I should like to mention two more experiments. The first of this is an experiment carried out on the Quaker Oats farm. Here two lots of dairy cows were fed a typical winter ration consisting of hay, silage, and grain. One lot was fed forage juice at a level of 2% of the grain mixture. In both cases the grain was fed at a rate of 1 lb./4 lbs. milk produced. The forage juice fed animals gave an average production of 9,040 pounds of milk per lactation as compared with the control average of 7,640 pounds. The increase of over 18% was statistically significant.

I would now like to discuss the results of some work at the University of Wisconsin on European Corn Borer. (Chart 11) Forage juice contains a factor which stimulates larval and pupa weight and is essential for maturation. We do not want to grow more corn borers but they may prove to be a valuable research tool.

So much for the biological work on forage juice. I would now like to turn to processing methods which have been used. A consideration of the structure of a leaf makes it apparent why leaf fractionation is more difficult than grain milling. In grain the fiber is almost entirely in layers on the outside of the kernel. The desired fraction, the flour, is concentrated in the endosperm of the seed. In contrast, leaf fiber is present as the cell wall in every cell of the leaf. The desired nutrients are the cell contents of every cell. In the leaf we must fractionate cells instead of large organs like wheat kernels.

Two methods have been used. The first consists of grinding the leaf in a vertical hammermill to break as many cells as possible. The pulp is then pressed in a horizontal type screw press (tapered screw core and conical plug). This type of press gives high pressure and maximum shear so that cell contents are removed including protein as well as water-soluble components. The green juice obtained is concentrated in a vacuum evaporator modified to give a high velocity of liquid across the heating surface. The limit to which this high protein type of juice can be concentrated is to about 35% solids. Above this point the viscosity increases rapidly and evaporator tubes foul to a serious degree. The 35% solids concentrate is not stable at room temperatures unless preservatives are added. If the green press juice is heated to 70 to 80° C. or acidified, a green protein fraction and a brown juice fraction are obtained. The separation of the protein is a difficult operation by methods thus far tried. The dried protein fraction which is rich in carotene, xanthophyll, vitamin E and vitamin K might well find use in poultry feeds. The brown juice fraction can be concentrated to 50 to 60% solids to give a stable product rich in the unidentified growth factors described above.

The second method of preparing a stable brown juice concentrate consists in heating the chopped fresh forage in a countercurrent extractor similar to that used in the beet sugar industry. The heat coagulates the protein within the cells and alters the cell permeability so that the unidentified growth factors, the B vitamins, and other water-soluble nutrients can diffuse through the cell wall. The press used for this type of operation is one which does not apply a shearing action on the extracted residue. The latter may be dehydrated in a conventional drum dehydrator. The concentrated brown juice is comparable to that described above. This type of juice product was used in essentially all of the biological work reported with the exception that an

"activation" step was added which involved addition of copper sulfate to the juice (.5 to 1% of the juice solids). Earlier work had shown that this treatment improved somewhat the growth responses obtained in chicks and turkey poults.

Several areas of research require additional work. On the fundamental side, the nature of the unidentified growth factors should be determined and improved assay procedures developed. On the processing side, methods of producing a potent dried juice should be developed. Process cost studies are also needed. Further work is needed on the use of the forage juice products in high-energy rations for poultry and swine and one use of the dehydrated meal fraction in ruminants.

The work to date indicates that the fractionation of forages has a great deal of promise for upgrading forage value. Capital expenditures will be relatively high but the basic process is inexpensive since evaporation in multiple effect evaporators requires less fuel than conventional dehydration.

CHART 1

EFFECT OF VITAMINS AND JUICE ON GROWTH OF CHICKS FED P-18

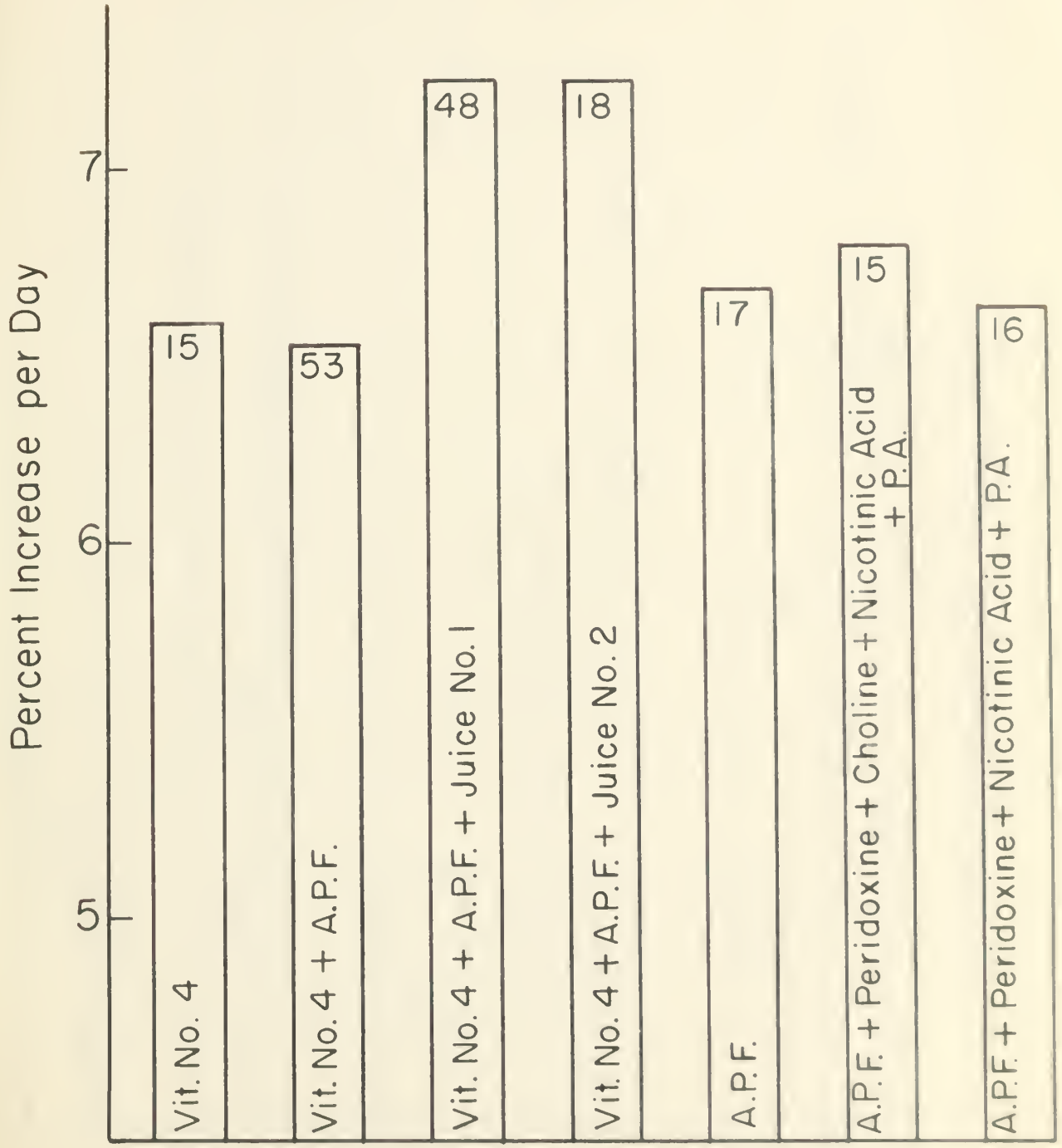


CHART 2

EFFECT OF DEHYDRATION ON THE GROWTH-PROMOTING PROPERTIES OF GRASS

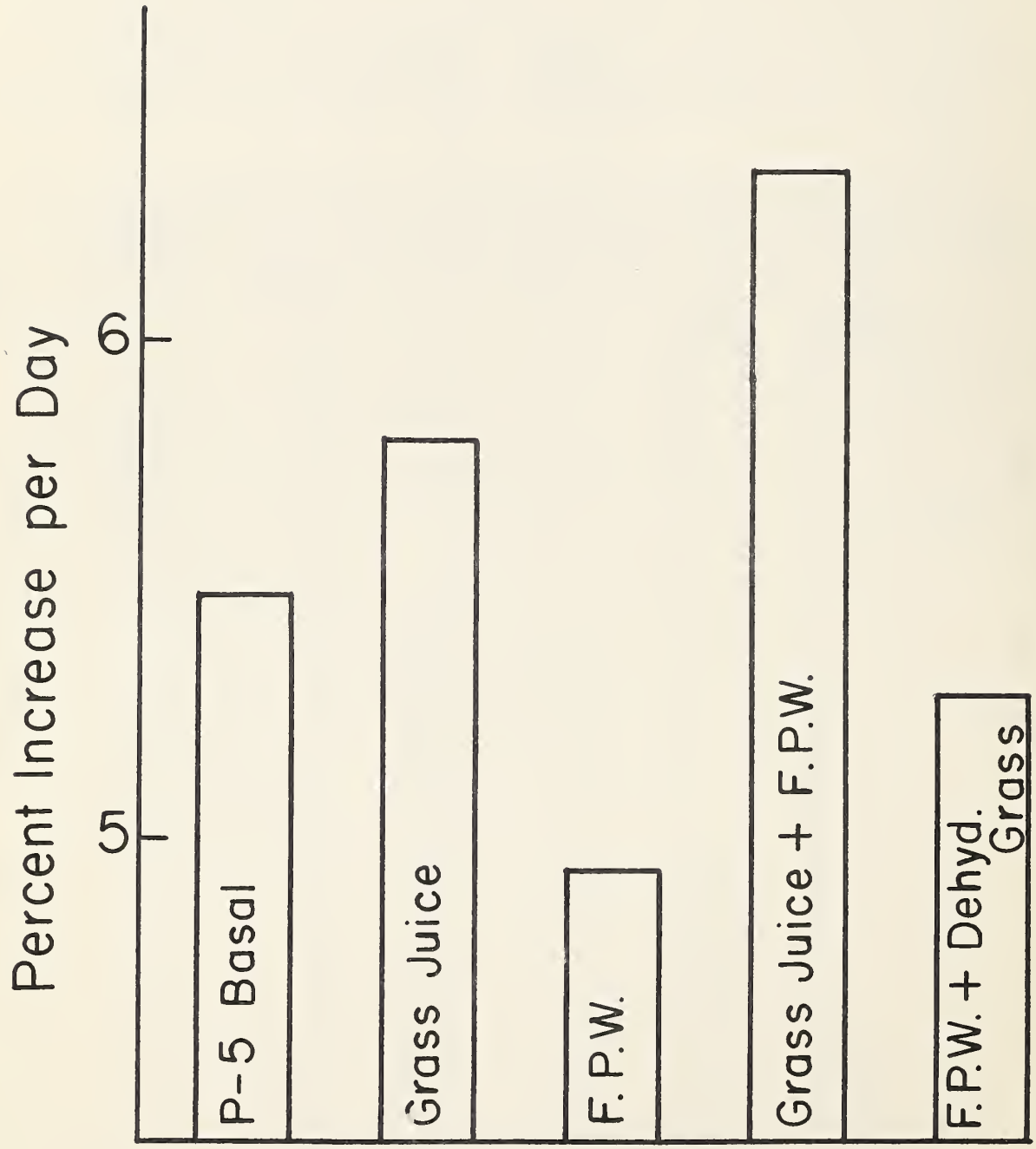


Chart 3. Effect of Drying Whole Forage Crops on Their Growth Factor Potency

Exp. No.	Basal Ration	Negative control basal alone (percent gain/day)	Positive control basal + 3% juice conc. (percent gain/day)	Basal + 3% dried forage crop		
				Drying procedure	Pretreatment	Percent gain/day
7132	P-5 + 2% Fish Solubles	4.95	6.35	Dehy.[1]	none--wheat	5.30
8279	P-14 + 2% Fish Solubles	5.89	6.50	Dehy.[1]	none--rye	5.47
8125	P-11 + 2% Fish Solubles Sol. + 2.5% dried whey	5.05	6.70	Dehy.[2]	none--alfalfa	5.65
9193	P-18 + 0.5% Led. APF	6.59	7.00	Oven dr.[3]	disintegrated-adj. to pH 9.0	6.72
	+500 % pyridoxal +0.05% protamone					

[1]Arnold Dehydrator--triple pass drum--commercial size--inlet temperature 1700-1800° F.--outlet temperature 250-280° F.

[2]Pilot scale--single pass drum dehydrator--inlet temperature 1200-1400° F.--outlet temperature 300-320° F.

[3]Dried at 100° C. for ca. 90 min. in a forced draft Procter-Schwartz tray drier.

CHART 4

SEASONAL RESPONSE OF CHICKS TO

GRASS JUICE AND FISH PRESS WATER

1948

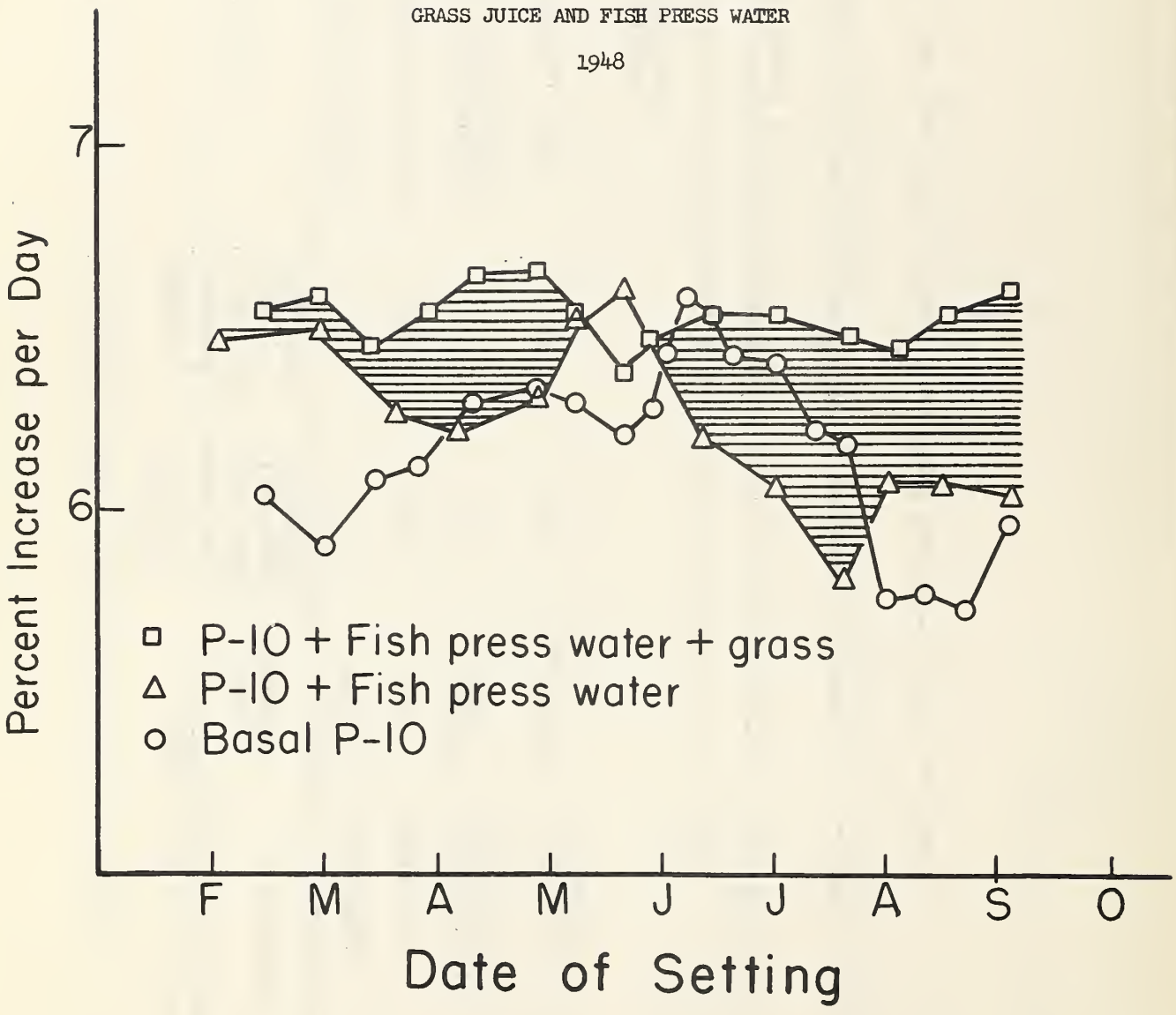
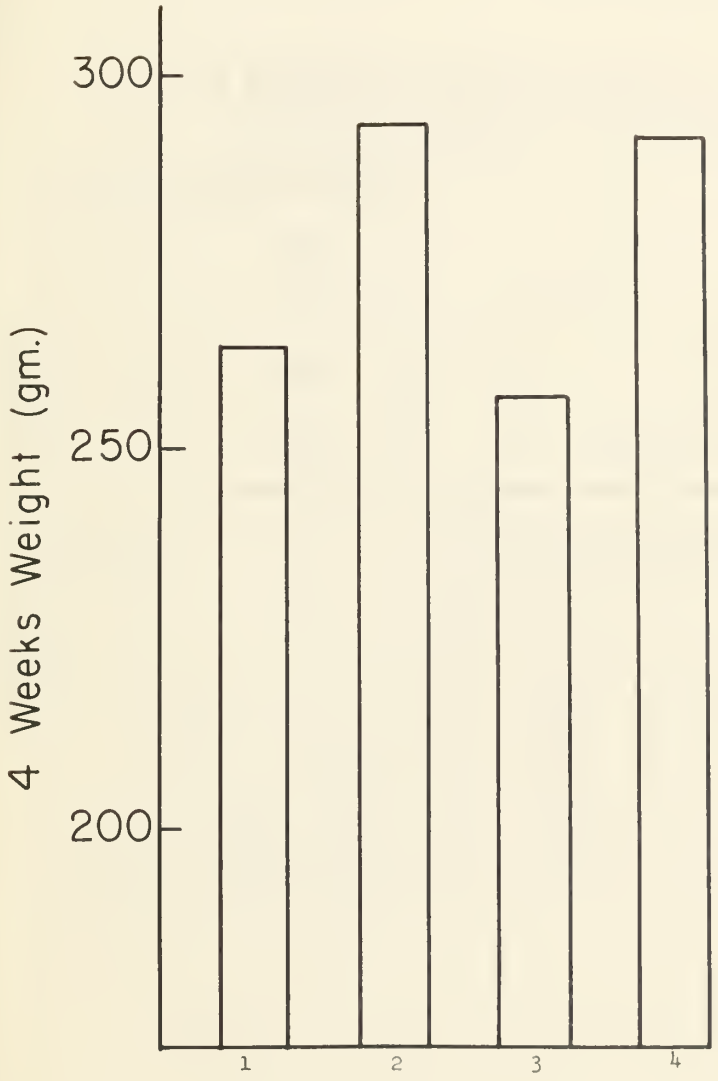


CHART 5

CORNELL - SCOTT, NORRIS, HEUSER AND HILL - REPORT JULY 1, 1953

Group 1 - Wh. leghorn males - Basal
2 - " " " - + 1.5% Juice
3 - Wh. rocks - mixed sex - Basal
4 - " " " " - + 1.5% Juice



Basal #2

Fish meal, menhaden	34.0 gm./100 gm. diet	
Cornstarch	61.34	
Cellophane	3.0	
Iodized salt	0.4	
KCl	0.4	
$MgSO_4$	0.25	
Nopcay (10,000 IU/gm.)	0.08	
Delsterol (1,500 IU/gm.)	0.02	
Choline Cl (70%)	0.29	
$FeSO_4 \cdot 7H_2O$	54.0	mg.
$MnSO_4 \cdot H_2O$	35.0	"
$ZnCl_2$	1.0	"
$CoCl_2 \cdot 5H_2O$	0.2	"
$CuSO_4 \cdot 5H_2O$	1.5	"
l-Inositol	100.0	"
α-Tocopherol (25%)	16.0	"
Niacin	5.0	"
Ca pantothenate	2.0	"
Menadione	1.0	"
Pyridoxine HCl	1.0	"
Riboflavin	1.0	"
Thiamine HCl	1.0	"
Folic acid	0.2	"
Biotin	0.02	"
B_{12}	0.002	"

Chart 6. Effect of alfalfa juice and aureomycin on growth of chicks fed glucose as the carbohydrate

Supplement	5 wk. wt. gm.
Basal	428
5% Alfalfa juice	413
Aureomycin, 10 mg./lb.	437
Alfalfa juice & aureomycin	494

Hill, et al., 1953

CHART 7

CORNELL UNIVERSITY - PROF. SCOTT
12/3/53

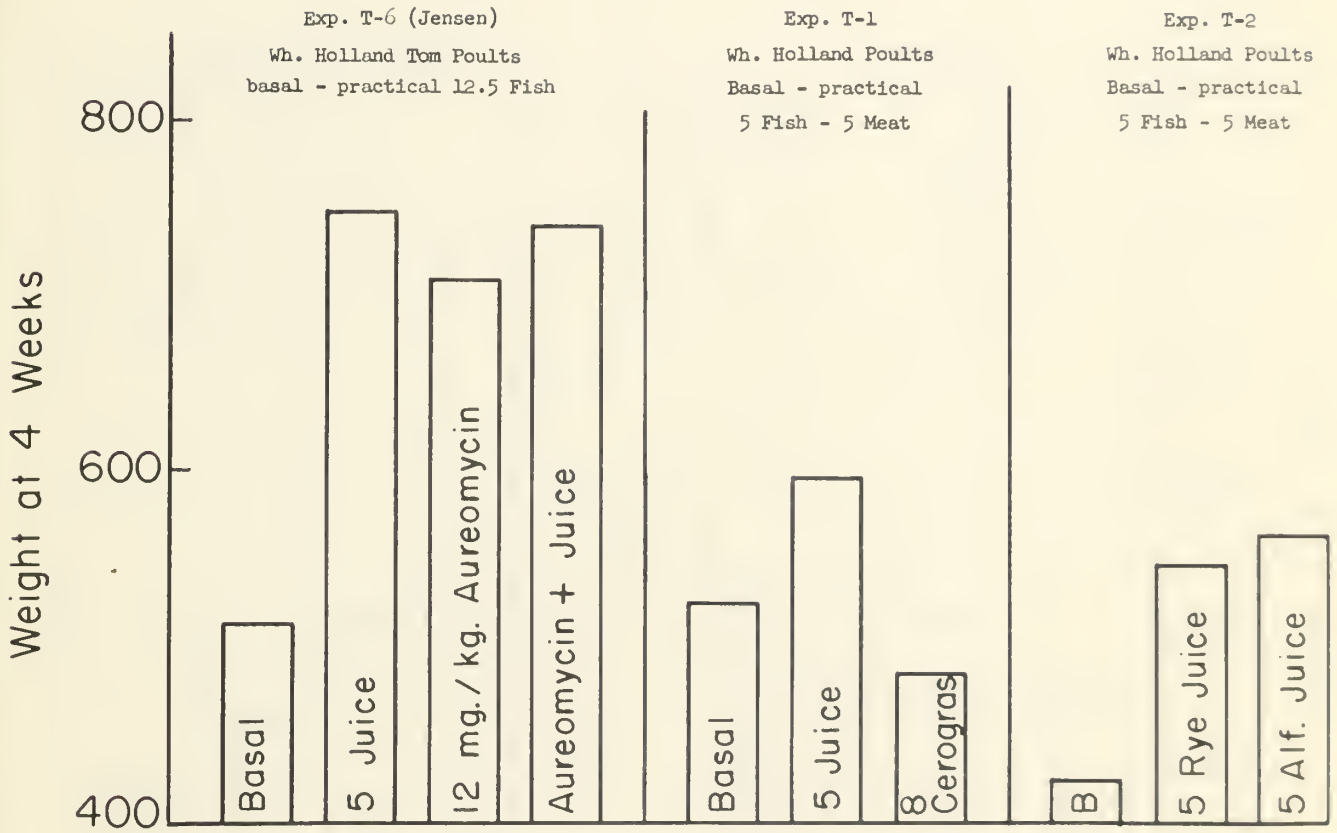


CHART 8

CORNELL UNIVERSITY - PROF. SCOTT
12/3/53

Exp. T-5 Broad Breasted Bronze

Basal - Practical - 12.5 fish

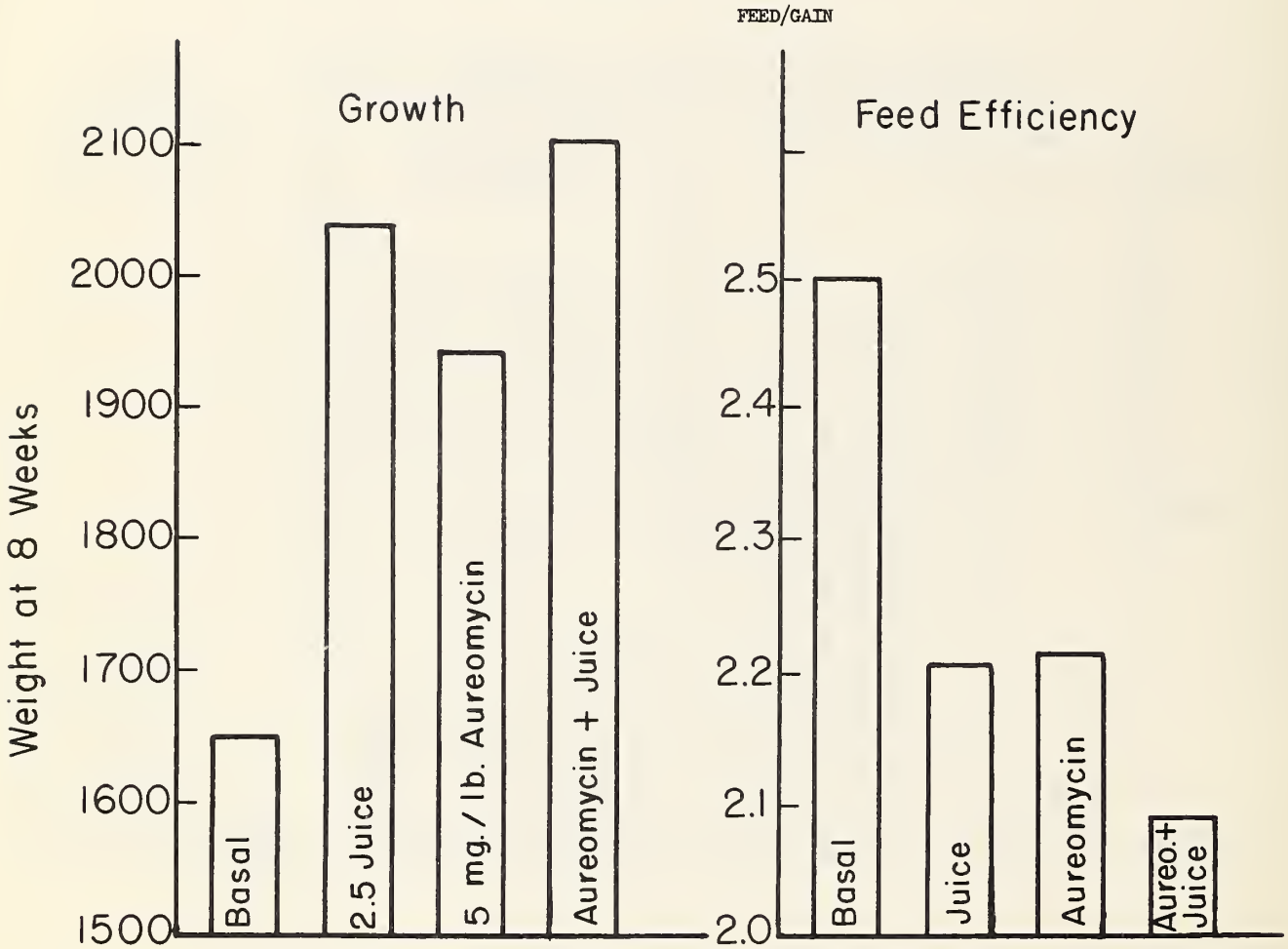


Chart 9

M. L. Scott, Cornell U.: Separate and Combined Effects of Forage Juice, Antibiotic, Methionine, and and Vitamin E on Poult. Growth

	Without Aurecmycin		With 12 p.p.m. Aureomycin	
	gm.		gm.	
Basal diet	396)		500)	
0.3% Methionine	542)	Avg. 491	588)	Avg. 556
20 mg./lb. Vitamin E	500)		538)	
Methionine + Vitamin E	524)		603)	
Basal + 5% Forage juice	566)		552)	
0.3 Methionine + 5% Forage juice	616)	Avg. 563	657)	Avg. 606
20 mg./lb. E + " " "	566)		624)	
Methionine + E + " " "	604)		592)	

Chart 10

Cornell - Carryover Experiment on Turkeys

Hen Diet	Poult Diet	Avg. Wt. 4 Wks.	Survivors	Mortality
		gms.		%
Basal	Basal	545	9/18	50
+ 5% Grass juice	Basal	579	17/18	6
Basal	+ 5% Grass juice	646	17/18	6
+ 5% Grass juice	+ 5% Grass juice	681	18/18	0
Basal	Commercial	609	3/36	8

Chart 11

Growth and Maturation of European Corn Borer Larvae on Diets Containing Different Sources of Vitamins

Dietary source of vitamins	Growth of larvae at 21 days		
	Avg. larval weight	Avg. pupal weight	Per cent mature
	mg.	mg.	mg.
Leaf concentrate plus vitamin mixture (basal)	65.4	94.1	83.3
Yeast powder only	22.1	43.0	31.2
Leaf concentrate only	21.1	--	0.0
Vitamin mixture only	15.6	--	0.0
Yeast powder plus vitamin mixture	22.6	47.5	17.6
Leaf concentrate plus yeast powder	80.3	77.3	82.6

FORAGE JUICE IN SWINE FEEDS

D. I. Gard

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Forage juice is a very fascinating research ingredient to study for unidentified nutritional factor(s). This is attested by the reports found in the literature on its favorable effects on growth and reproduction with poultry and swine. This paper will focus its attention on the use of forage juice in swine nutritional experiments with a few comments in reference to laboratory animals.

Forage juice is not a relatively new research ingredient. Over twenty years ago, Kohler, Elvehjem, and Hart (1936 and 1938) observed that young rats fed summer milk or winter milk supplemented with a daily allowance of 3 cc. of fresh clear grass juice gained twice as rapidly as rats fed the winter milk. The authors also observed that various grasses contain a factor(s) which is soluble in the plant juice. This factor was found essential for maintenance and growth of guinea pigs.

One of the earliest forage juice studies with swine was reported by Vestal, et al. (1949). Three percent alfalfa juice (1% dry matter) and 5% alfalfa meal were added to the control ration of young, 63-pound gilts and fed until a week after farrowing. The control ration consisted of corn, soybean meal, steamed bone meal, pulverized limestone, iodized salt and concentrated cod liver oil. Each treatment consisted of 14 gilts maintained in paved lots. During lactation, sows and pigs were fed a ration of corn, oats, protein and mineral supplements on Balbo rye pasture. Pigs were weaned at 56 days of age. The farrowing results are shown in Table 1.

With the forage materials in the growing and gestation ration, more gilts farrowed and they farrowed heavier, stronger pigs. However, the control gilts farrowed slightly more pigs per litter than the two forage treatments (8.5 vs. 8.0).

The lactation results are shown in Table 2. Although all sows and litters were maintained on Balbo rye pasture, the effect of previous treatment is evident in the weaning results. The sows that received alfalfa juice earlier in life weaned the most pigs--6.6; those that received alfalfa meal weaned 4.6 pigs; and the control sows weaned 2.8 pigs. Average pig weaning weights on all treatments were similar. Therefore, the average litter weaning weights vary directly with the number of pigs weaned. It is possible that a vitamin deficiency was the primary limiting factor in this study as indicated by the over-all poor gestation and lactation performance.

The first swine growth study with forage juice was reported by Wahlstrom (1951). Three percent grass juice concentrate was added to an α-protein "synthetic milk" ration known to be adequate in vitamin B_{12}. The ration including grass juice increased gains 8 percent.

Gard, et al. (1955) reported growth response with grass juice concentrate in a series of three 8-week experiments with pigs weighing about 25 pounds. A 2^3 factorial experimental design with four replicates involving 32 pigs was used in all experiments. In the first two experiments, the pigs were from dams that had been on purified diets. During these two growth tests, the test

ingredients were added to a fortified corn-starch isolated soybean protein diet. The pigs were individually fed ad libitum on screens. In the third experiments, the pigs were from dams fed a practical diet on pasture. The test ingredients were added to a fortified corn-soybean meal diet. The pigs were group-fed ad libitum on concrete. The ingredients tested and the percent added to the diets in the three experiments is shown in Table 3.

The composition of the control diets for these experiments is given in Tables 4 and 5. All diets were equalized for crude protein at 16 percent. The preparation of the grass juice was described by Kohler and Graham (1951). This material contained 50.0 percent solids, 5.0 percent protein, and 12.5 percent ash.

Final weights were analyzed by covariance in each experiment to adjust for differences in initial weight. Feed consumption data in the first two experiments were analyzed by covariance to adjust for differences in initial weight. In these same two experiments, final weights were analyzed by covariance to adjust for differences in feed consumption in addition to differences in initial weight.

The pertinent adjusted average daily gains for the first experiment are shown in Table 6. Grass juice concentrate and dried whey each increased average daily gain significantly ($P < 0.05$) over that of pigs receiving neither, but their effects were not additive and pigs receiving both did not gain significantly better than those receiving either one.

Thus, the grass juice concentrate and dried whey interaction was found significant ($P < 0.05$). The pigs receiving grass juice concentrate consumed significantly ($P < 0.05$) more feed than pigs receiving no grass juice concentrate. When final weights were also adjusted for differences in feed consumption, the grass juice concentrate growth response approached significance.

In the second and third experiments, the pigs receiving grass juice concentrate made significantly ($P < 0.01$) faster gains than the pigs receiving no grass juice concentrate as shown in Table 7. In the second experiment, the pigs receiving grass juice concentrate consumed significantly ($P < 0.01$) more feed than the pigs receiving no grass juice concentrate (2.97 vs. 2.65 lb. daily). When a multivariate test was made, the growth response was still significant at the five percent level.

Since Legg, et al. (1950, and Cheng, et al. (1952), observed that grasses and legumes showed estrogenic activity and Burroughs, et al. (1954), had observed that diethylstilbestrol in the diet of steers produced a marked improvement in growth and feed conversion, it was postulated that the pig's growth response to the grass juice concentrate might be due to its estrogenic content. Therefore, the grass juice concentrate was assayed for estrogenic activity by the rat uterine weight method, using one-month-old castrate female rats. A standard dose-response curve was established with estradiol benzoate to compare with the dose response of grass juice at various levels. The maximum uterine growth response produced by the grass juice concentrate indicated that 1 ml. contained no more than the equivalent of 0.016 mg. of estradiol benzoate. Thus, in experiments where 3 percent grass juice concentrate was included in the rations, the pigs receiving grass juice concentrate may have consumed the approximate equivalent of 0.7 mg. of estradiol benzoate daily. This level related to body weight agrees with the levels used in ruminant tests.

Conrad and Beeson (1957) studied several sources of unidentified growth factors with various levels of minerals using a "semi-purified" diet. Early weaned pigs--21 days of age and weighing about ten pounds--were fed for a period of 40 or 70 days. The pigs were fed in pairs in small concrete floor pens with feed and water supplied ad libitum. The 3% grass juice concentrate and additional trace minerals were added to the 20% control ration as shown in Table 8. When the pigs averaged 40 pounds, the protein content of the ration was decreased to 16%. Each experimental treatment consisted of 2 Durocs and 4 Chester Whites. The Chester Whites were started 10 days later than the Durocs. Final weights were statistically analyzed.

The 70-day results with the Duroc pigs are shown in Table 9. During the experiment, some of the pigs failed to grow. Later a skin condition appeared which was diagnosed as parakeratosis. Fifty-six days after the start of the experiment, several changes were made in the diets: Calcium reduced from 1.06% to 0.68%; phosphorus reduced from 0.68% to 0.58%; and total zinc increased from 28 p.p.m. to 72 p.p.m. Growth rate of the pigs receiving 3% grass juice concentrate and additional trace minerals was considerably improved over those fed the semi-purified control ration.

The 40-day results with the Chester White pigs are shown in Table 10. Three weeks after the start of the experiment, they developed a skin rash which was later diagnosed as parakeratosis. Under these conditions grass juice concentrate stimulated gains and gave definite protection against parakeratosis.

SUMMARY

Forage juice concentrate has been tested for its unidentified nutritional factor(s) activity in five swine growth experiments and a reproduction experiment. Under the conditions of each experiment, forage juice concentrate consistently improved growth or the reproduction performance. A forage juice sample was assayed for estrogenic activity by the rat uterine weight method. It was indicated that 1 ml. of forage juice concentrate contained no more than the equivalent of 0.016 mg. of estradiol benzoate. Whether any of the nutritional response can be attributed to its estrogenic activity has not been defined to date. It has been postulated that some of its growth effect may be attributed to its trace mineral content.

Table 1

Farrowing Data
Purdue, 1949

	Materials Tested		
Items Compared	None	3% Alfalfa Juice	5% Alfalfa Meal
No. Bred	14	14	14
No. Farrowed	11	13	13
No. Pigs/Litter	8.5	8.0	8.0
Avg. Birth Wt./Pig, Lb.	2.15	2.28	2.21
% Pigs Farrowed:			
Strong	42	57	47
Dead	4	9	3

Table 2

Weaning Data
Purdue, 1949

Items Compared	Materials Tested: None	3% Alfalfa Juice	5% Alfalfa Meal
No. Litters Weaned	8	9	12
Avg. No. Pigs Weaned/Litter	2.8	6.6	4.6
Avg. 56-Day Pig Wt., Lb.	26.4	25.2	24.0
Avg. 56-Day Litter Wt., Lb.	74	166	110
% of Pigs Weaned	23	57	49

Table 3

Ingredients Tested in Growth Tests
Illinois, 1955

Ingredients	Experiment No. 1	2	3
	%	%	%
Menhaden Fish Solubles	-	3	3
Grass Juice Concentrate	3	3	3
Dried Whey Product With Whey Fermentation Solubles	4	5	5
Dried Brewers Yeast	10	-	-

Table 4.

Composition of Purified Diet
Illinois, 1955

Ingredients	Percent
Corn Starch	60.3
Drackett Protein 220	19.7
Dextrose	10.0
Woodflock	3.0
Corn Oil	2.0
Vitamin A and D Oil (3000A, 600D)	0.5
DL-methionine	0.5
Mineral Mixture No. 2[1]	4.0
Vitamin Mixture No. 46[2]	+
Total	100.00

[1]The percentage composition of mineral mixture No. 2 was as follows: $CaHPO_4$ 65.0, iodized NaCl 16.0, K_2CO_3 14.0, $MgCO_3$ 3.43, $FeSO_4.7H_2O$ 1.00, $MnSO_4.H_2O$ 0.30, $CoCl_2.6H_2O$ 0.10, $CuSO_4$ 0.10, NaF 0.02, $ZnCO_3$ 0.04, and KI 0.01.

[2]Vitamins added per pound of diet: (mg.) thiamine HCL 1.5, riboflavin 3.0, calcium pantothenate 15.0, niacin 20.0, folic acid 0.5, pyridoxine HCL 1.5, vitamin B_{12} 0.015, biotin 0.03, p-aminobenzoic acid 10.0, inositol 90.0, α-tocopherol acetate 5.0, 2-methyl-1, 4-naphthoquinone 0.9, and choline chloride 600.0.

Table 5.

Composition of Practical Diet
Illinois, 1955

Ingredients	Percent
Ground Yellow Corn	74.4
Solvent Soybean Meal (45.0%)	22.4
Steamed Bone Meal	1.6
Ground Limestone	0.8
Salt (Iodized)	0.5
Trace Minerals[1]	0.1
Vitamin A and D Conc. (5000A-750D)	0.2
Vitamin Mixture No. 37[2]	+
Total	100.00

[1]Added to the ration (p.p.m.): Mg, 115; Fe, 40; Mn, 36; Co, 5; Cu, 8; Zn, 4.

[2]Vitamins added, mg. per pound of diet: Thiamine HCL 0.5, riboflavin 1.4, niacin 5.0, calcium pantothenate 4.5, pyridoxine HCL 0.6, folic acid 0.35, inositol 90.0, p-aminobenzoic acid 10.0, biotin 0.03, vitamin B_{12} 0.005, and choline chloride 454.

Table 6.

Effect of Grass Juice Concentrate and Dried Whey on Daily Gains in Lb. for Experiment 1
Illinois, 1955

Grass Juice Concentrate	Dried Whey		
	-	+	Avg.
-	0.98	1.19	1.08
+	1.22	1.25	1.24
Average	1.10	1.22	1.16

Table 7

Adjusted Average Daily Gain in Lbs.
For Experiments 2 and 3
Illinois, 1955

	Grass Juice Concentrate	No Grass Juice Concentrate
Experiment 2	1.16[1,2]	1.00
Experiment 3	1.34[1]	1.10

[1]$P < 0.01$ when gains adjusted for difference in initial weight.

[2]$P < 0.05$ when gains also adjusted for differences in feed consumption.

Table 8

Composition of Semi-Purified Ration
Purdue, 1957

Ingredients	Percent
Corn Starch	31.37
Glucose (Cerelose)	31.38
Isolated Soybean Protein	25.00
Corn Oil (Mazola)	5.00
Celluflour	3.00
DL-methionine	0.25
Mineral Mixture[1]	4.0
Trace Mineral Mixture[2]	+
Vitamin Mixture[3]	+
Antibiotic[4]	+

[1]Supplied the following percent of the ration: Iodized NaCl, 0.5; $CaHPO_4$, 2.0; $CaCO_3$, 1.5; KCl, 0.5; and KH_2PO_4, 0.5.

[2]Grams added per 100 pound of ration: $MgSO_4 \cdot 3H_2O$, 100; $Fe_4(P_2O_7)_3 \cdot 9H_2O$, 40; $MnCl_2 \cdot 4H_2O$, 6; $CuSO_4 \cdot 5H_2O$, 2; CaF_2, 1; $CoSO_4 \cdot 7H_2O$, 0.1; and ZnO, 0.6.

[3]Vitamins added, mg. per lb. of diet: Thiamine HCL, 3.0; riboflavin, 4.0; niacin, 15.0; calcium pantothenate, 10.0; pyridoxine HCL, 1.5; choline chloride, 600; para-amino-benzoic acid, 4.0; biotin, 0.1; inositol, 50; folic acid, 2.0; ascorbic acid, 50; mixed tocopherols, 15.0; 2-methyl-1,4-naphthoquinone, 1.0. Other vitamins added per lb.: Vitamin B_{12}, 20 micrograms; vitamin A acetate, 2,000 IU; vitamin D_2, 200 IU and vitamin D_3; 200 IU.

[4]Chlortetracylcine HCL was added to supply 25 mg. per lb.

Table 9

70-Day Duroc Growth Data
Purdue, 1957

Materials Tested	Phase 1	Phase 2	
		Effect of Ca and Zn Adjustment	
	56 Days	14 Days Before	14 Days After
	A.D.G.	A.D.G.	A.D.G.
None	0.63	0.16	0.84
Trace Minerals 1.5 X Control	0.95	0.95	1.59
3% Grass Juice Concentrate	0.91	1.03	1.45

Table 10

40-Day Chester White Growth Data
Purdue, 1957

Materials Tested	Avg. Daily Gain	Feed/ Lb. Gain	No. Para-keratotic Pigs
None	0.62	2.47	3
Trace Minerals 1.5 X Control	0.74	2.24	2
3% Grass Juice Concentrate	1.00	2.25	1

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THE CHEMISTRY AND DISTRIBUTION OF ESTROGENS IN FORAGES

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In earlier presentations today, Dr. Copley, Joe Chrisman, and Al Booth have all referred to the potentialities of a properly blended high-coumestrol alfalfa meal as a possible substitute for stilbestrol in beef cattle production. For my part of the program this afternoon, I should like to describe briefly the estrogens that occur in green forages and show their structural relationship to stilbestrol and to the natural animal estrogens. I should then like to point out the large variation in estrogen content that occurs in forages and indicate some of the factors that may cause this variation. Next, I should like to tell you of the progress we have made on the development of a chemical method for coumestrol, and finally, I would like to indicate some of the problems that have to be solved before it will be possible to produce a standardized high-estrogen alfalfa meal.

Genistein (Figure 1) was the first estrogenic compound to be isolated from forages. It was discovered in subterranean clover by workers in Australia. G nistin, the glycoside of genistein, had earlier been found in soybean meal by Dr. Walter of our own Laboratory.

Daidzein, a related estrogenic compound, has also been found as a glycoside in soybean meal but not as yet in forages. Daidzein is closely related to genistein, differing only in the absence of hydroxyl group at position 5 in the molecule. Subsequently, formononetin and biochanin A, two other closely related isoflavones, have been found in subterranean clover and these two estrogens together with genistein, have also been found in red clover.

It is interesting to compare the structural formulas of these isoflavonoid estrogens with that of stilbestrol (Figure 1). It is undoubtedly this similarity in structure that contributes to their activity. The estrogen, coumestrol (Figure 1), was first isolated at this Laboratory and the structure determined in 1957. It was because of the coumarin configuration in the molecule that we decided on the name coumestrol. Coumarins differ from isoflavones by having a double bonded oxygen atom attached at the 2 position rather than the 4 position on the molecule. Coumestrol is more than 30 times as estrogenic as genistein or the other isoflavones, but still considerably less active than stilbestrol. We have found coumestrol in all leguminous plants tested to date, including samples of frozen peas and green beans.

It would appear that the distribution of coumestrol in legumes is quite widespread, although the concentration in many plants may not reach a level capable of causing a physiological response. Of the several forage estrogens, coumestrol appears to predominate in alfalfa and ladino clover. Workers at Purdue University found that individual samples of sun-cured and dehydrated alfalfa meals varied more than 30 fold in estrogen content. Professor Kitts at the University of British Columbia reports stilbestrol equivalencies of alfalfa samples ranging from almost nil to as high as 55 micrograms per pound of dry matter. There are very wide differences in commercial meals, some showing no activity at all while others are quite estrogenic. All the possible reasons

for these wide variations are not known. One of the causes of variation may be the actual processing operation itself. We have obtained evidence in our Laboratory that variable amounts of estrogenic activity may be destroyed during the drying process. Another factor affecting estrogen content is probably the rate of loss during storage of the meal. Mr. Swierstra, in Professor Kitt's Laboratory in Canada, reported in 1958 that alfalfa and ladino clover meals may lose most, if not all, of their estrogenic activity after 6 months' storage at room temperature. It is interesting to note that the estrogenic activity of the feed extract mixture used in the mouse assay also tended to decrease but at a much slower rate. Similar results were obtained with ladino clover, but samples of red clover were much more stable. Genistein, which is the predominant estrogen of red clover, appears to be more stable than coumestrol.

In storage stability studies at our Laboratory, we have tended to confirm the observations on the instability of the estrogen in alfalfa and ladino clover, but our results suggest that the loss may not be as great as reported by Swierstra. In some cases, we found little or no loss after several months' storage. Variable amounts of loss occurred in other cases.

Other possible reasons for estrogen variation in forages are stage of maturity, frequency of cutting, time of year, location of stand, variety of alfalfa, age of stand, and fertilization treatment. As early as 1949, Dr. S. P. Legg at the National Institute for Research in Dairying, Reading, England, had made a study on the seasonal and species distribution of estrogens in British pasture plants. His results clearly showed that there was considerable seasonal variation. At least with the grasses, their results suggest that high estrogen concentration is associated with the period of reproductive growth, which occurs in the spring. Then, when autumn growth takes place, the plant is no longer in a reproductive phase and estrogen content is low. Broad red clover, on the other hand, will produce flowers throughout the growing season.

In 1956, Professor Andrews of Purdue University reported their studies on the estrogenic content of alfalfa at different stages of maturity. They found that the activity varied widely in the first spring crop. During the vegetative stage no activity was present but it suddenly appeared at the very early budding stage. At the full budding stage the estrogen again disappeared. Following the beginning of bloom, there was a gradual increase in activity which reached a maximum at full bloom. The activity remained relatively high from 1/4 bloom through the seedhead stage. These results confirm the earlier British observations that high estrogen concentration is associated with the reproductive growth of the plant. At Purdue, the pattern of estrogenic activity during the second, third and fourth crops was considerably different from that of the original spring growth. The second cutting did not show the large increase during early budding and did not become markedly estrogenic until the dough stage was reached. Estrogen content remained low throughout the entire life cycle of the plant during both the third and fourth cuttings.

At the University of British Columbia, Professor Kitts and his students have recently completed a somewhat similar study (see Table 1). They found that the estrogenic content was highest at the vegetative stage, tending to decrease until full bloom, and then increasing again in late bloom, when the plants are partially in the dough stage. They concluded from these results that estrogenic activity was not associated solely with the phase of rapid growth nor with the reproductive phase of the plant. In a related portion of their study, four successive cuttings of alfalfa were made throughout the summer, all plants being

harvested at almost the same stage of development. The estrogenic content was high in the spring, tending to decrease during the summer but building up again in the fall. This work is in disagreement with the findings of the Purdue group that alfalfa cuttings subsequent to the first tend to be lower in estrogenic activity. However, it should be pointed out that these apparently divergent results may indicate real differences due to climate, locality, soil conditions or other factors.

At this Laboratory, we have just completed a similar study for 5 successive crops in 1958 and the first growth of the 1959 season. Our results confirm the findings of the Purdue workers for the first growth, showing little estrogenic activity until about 1/4 bloom, after which a rapid and progressive increase in estrogen content occurred and continued throughout the life cycle of the plant. However, contrary to the Purdue findings we find a similar gradual buildup of estrogen content during each of the six cuttings studied. I should like to emphasize that we do not find the estrogen content to be highest at the immature stage, when alfalfa is customarily harvested for dehydration. Rather, peak estrogen content appears at about full bloom or dough state, when the plant is past its prime from the standpoint of prime quality dehydrated meal production. In a cooperative study with Professor Roubicek of the Arizona Experiment Station, a series of sun-cured meals were prepared and sent to this Laboratory for assay. The samples were taken from different fields and represent average cuttings during the month period involved. The first two cuttings showed almost no activity. The estrogen content was extremely nigh during the January growing period and then gradually tapered off in later months. Perhaps these results suggest the importance of temperature and length of daylight on estrogenic content in the plant.

In order to gain some insight into the influence of varietal differences on estrogen content, a study was recently made at the Purdue Agronomy Nursery of 56 strains of alfalfa. All the plants were harvested in the bud stage from second cuttings. The study included such common varieties as Ranger, Grimm, Hardigan, Cossack, Buffalo, and Viking. There were extremely wide differences in the estrogenic potencies of different samples, even in clones or strains from the same alfalfa varieties. Current studies now under way at the Purdue Station include variations within individual clones and selection for estrogen content.

In this brief review, I have tried to indicate some of the problems that await solution. It is obvious that the work that has been done to date has barely scratched the surface in indicating the directions in which further studies might profitably be carried out. In order to expedite such studies, it is necessary that a rapid, accurate, chemical assay be developed to replace the rather slow and expensive bioassay procedure now employed for estrogen evaluation. For approximately the past year we have been working on the development of such a method and we feel that we are fairly close to its accomplishment.

In closing, I should like to point out again that a possible curtailment of the use of stilbestrol for beef production might provide the stimulus for greatly expanded use of high-estrogen alfalfa meal. However, this expanded use is dependent upon proof that alfalfa meal can substitute for stilbestrol as a growth promoter.

Some cooperative animal studies are now under way in order to clarify this point. Professor Matsushima at Nebraska is comparing a high- and low-estrogen-containing alfalfa meal for beef cattle production; Professor Oldfield of Oregon State is studying the same two meals for sheep production; and Dr. Sykes of the

U. S. Department of Agriculture is determining their effectiveness for increased milk production in dairy cattle. We appreciate the cooperation of the American Dehydrators Association in helping to make these meals available, and we hope to have the results of these studies later this summer.

If these results are promising and if alfalfa is to be used for this purpose, then we must have information which will assure the preparation and maintenance of high-potency stable meals. In anticipation of this eventuality and in order to be better able to exploit this possibility, we feel that the estrogen program at this Laboratory should proceed along the following lines:

(1) The further investigation of the production variables which need to be controlled in order to produce a consistently high-quality raw material. It may be possible by selection to produce alfalfa which contains more estrogen or other growth-promoting properties. It may well be that part of the unknown growth factors in alfalfa are associated with its hormonal activity.

(2) Simultaneous with this study should be an investigation of the processing variables in the production of dehydrated meal so as to keep estrogen losses to a minimum during processing. It would also be desirable to investigate other means of forage processing to prepare estrogen-rich concentrates.

(3) Since the estrogenic factors appear to be somewhat unstable, and perhaps susceptible to oxidation during storage, the use of inert gas storage and application of antioxidants should be investigated as a means of stabilizing the dehydrated product. The development of the rapid, analytical method along the lines that I described should help materially in these investigations.

Table 1

ESTROGENIC ACTIVITY OF FIRST CUTTINGS OF ALFALFA AT DIFFERENT STAGES OF MATURITY (VANCOUVER)

Date of Cutting	Stage of Maturity	Estimated* Potency
		mcgm.
May 3	Vegetative	55
June 3	Prebloom	12
July 2	Full bloom	4
Aug. 1	Late bloom	21
Sept. 3	Past bloom	17

*Calculated as stilbestrol equivalents per pound of dry matter.

Table 2

ESTROGENIC ACTIVITY OF 1ST, 2ND, 3RD, AND 4TH CUTTINGS OF ALFALFA (VANCOUVER, 1957)

Cuttings	Date of Cutting	Stage of Maturity	Estimated Potency*
			mcgm.
1	May 3	Vegetative	55
2	June 2	Vegetative	14
3	July 2	Vegetative	6
4	Aug. 15	Vegetative	43

*Calculated as stilbestrol equivalents per pound of dry matter.

THE PAST, PRESENT AND FUTURE OF ESTROGENS IN FEEDS

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The term "estrogen," as it is commonly used today, is a collective noun which includes all compounds that are capable of causing sexual development in the female including changes in the vaginal epithelium, hypertrophy of the uterus and mammary glands, and development of the secondary sex characteristics. Twenty years ago most of the compounds with estrogenic properties were chemically related to the animal steroids. However, in 1938 the first of three significant events in the area of basic research on estrogens took place with the discovery that a nonsteroid compound called diethylstilbestrol was estrogenic. For the first time an inexpensive, synthetic estrogen was available for clinical and experimental use. As we all know, in 1954 its use as a feed additive was approved and a patent covering such use was granted to the Iowa State College.

The second significant development in estrogen research came about during the period from 1946 to 1951 when the cause of infertility in sheep grazed on subterranean clover in Australia was traced to an estrogen called genistein. Several other flavonoids related to genistein have now been identified in various feedstuffs including forages and have likewise been shown to be weakly estrogenic.

Recently here at the Western Regional Laboratory we have successfully isolated and identified from forages a coumarin-like compound having estrogenic properties. This compound, to which the name coumestrol has been given, is more potent on a weight basis than the estrogen genistein, but considerably less potent than diethylstilbestrol.

Here in this laboratory a great deal of effort has now been expended on the most suitable means of measuring the amount of estrogenic activity in a forage sample. The major problem concerns the inability of the immature mouse to ingest large amounts of forage in the diet (see Table 1), thus making it necessary to extract the estrogen from the forage sample with solvents such as acetone. The acetone-insoluble fraction containing the crude fiber is discarded. The acetone-soluble fraction containing the estrogen is added to the special nonestrogen containing basal diet and is then fed to the mice after evaporation of the acetone. Weanling albino female mice are purchased locally and caged in groups of five for the bioassay. When a food allowance of 10 grams per mouse has been ingested which usually takes from 5 to 7 days, the animals are autopsied and the weights of the freshly excised uteri determined. Any increase in the group average uterine weight compared to the control group is taken as a measure of the estrogenic activity of the material being tested. An example of the direct proportionality between the uterine weights response and dosage of coumestrol is shown in Table 2. Five thousand mice per year are presently being used here for estrogen bioassay testing.

A comparison of the effects of various feed additives for fattening cattle including antibiotics, estrogens, tranquilizers, and anti-thyroid substances are shown in Table 3. The superiority of stilbestrol is clearly indicated. The positive effects of tapazole, a thyroid-depressing substance is interesting because it has been shown that stilbestrol stimulates the anterior pituitary resulting in a greater production of growth hormone but at the same time depressing the output of the thyroid hormone.

A summary of several experiments involving the feeding of stilbestrol to cattle is shown in Table 4. In most cases a positive weight gain was noted. In three instances no gain resulted. At the time these feeding experiments were conducted data on the estrogenic activity of the basal rations was not obtained. The possibility is advanced that perhaps in those instances where no response to added stilbestrol was observed, that the basal ration contained a sufficient level of plant estrogens to enhance growth. A further growth response from added stilbestrol would not then be expected. One of the most positive evidences of weight gain stimulation in steers by alfalfa is shown in Table 5. Here it can be seen that a daily intake of five pounds of alfalfa produced the same daily weight gain in pounds (2.46) as stilbestrol (2.47). Here again the estrogenic activity of the alfalfa used was not determined but experiments are in progress to clarify this point, by comparing a low-vs. a high-estrogen-containing alfalfa on the growth of steers. Even if estrogen content and growth rate do not correlate, the fact remains that alfalfa contains a growth-stimulating substance(s) which should be investigated.

Turning to other animal species, data on the effect of stilbestrol on cockerels are shown in Table 6. The major effect other than atrophy of the testes and comb is an increased deposition of body fat along with an increased food intake. No consistent growth stimulation has been observed when estrogen is fed to growing pigs. Many reports show that stilbestrol definitely stimulates live weight gains and improved feed conversion when fed to lambs.

Here at the Western Regional Laboratory experiments on the effect of coumestrol on the growth and reproduction of rats and mice have been investigated. Once again species differences were encountered in that no effects were observed when coumestrol was fed to male rats, whereas measurable increases in growth were observed when male mice received coumestrol in the diet. It was also possible to inhibit reproduction in mice, but not rats, when coumestrol was fed.

In summary, as more and more research data on the effects of feeding plant estrogens to animals accumulate, it is becoming increasingly clear that practical benefits from the use of these estrogens will be realized in the near future.

Table 1

Uterine Response of Immature Female Mice to Oral Administration of Ladino Clover Meal Admixed with Standard Diet

Clover Meal in Diet	Mean Uterine Weight
Percent	mg.
0	8
15	15
20	14
40	16
60	16

Table 2

Uterine Response to Graded Levels of Estrogen

Diet	Quantity Fed Per Mouse	Uterine Weight ± S.E.
	micrograms	mg.
Addis-Control Diet	---	9.73 ± 0.33
Coumestrol	100	13.74 ± 0.73
"	200	18.74 ± 0.97
"	300	25.68 ± 1.05
"	400	34.39 ± 1.23

S. E. = Standard error of mean.
No. of animals = 15 - 20/group.

Table 3

Feed Additive Usage in Supplements for Fattening Cattle
(Summary Based Upon College Experiments Reported to Date)

Feed Additive	Daily Amount Per Animal	Years Tested in Cattle Expt.	No. Colleges Conducting Expt.	Avg. Wt. Increase	Avg. Total Feed Saved	Additive(s) on Market	Benefits to Cattle Industry
Antiobiotics	75 mg.	9	15	4%	3%	Yes	Fair
Stilbestrol	10 mg.	6	20	18%	12%	Yes	Excellent
Live rumen cultures	?	5	4	?	?	Yes	Poor
Chemobiotics	?	3	8	?	?	Yes	Poor
Tranquilizers	?	2	5	?	?	Yes	Fair
Tapazole (35-80 days)	600 mg.	2	2	11%	6%	No	Good
Alcohol	1-3 oz.	1	3	?	?	Yes	Poor
Enzymes (dry corn rations)	0075 lb.	1	1	12%	7%	Poultry feeds only	Good

(From Wise-Burroughs, Iowa State College - Distillers Feed Conf. Proceedings, March 25, 1959, Cincinnati, Ohio, Vol. 14, page 89)

Table 4

Stilbestrol Benefits in Drylot (Growing) Rations

College Experiment	No. Days on Test	% Gain Stimulation	% Feed Saving
Iowa, Apr. 1954	127	9	10
" June 1954	75	16	9
" " "	168	16	25
Tennessee, Jan. 1955	171	8	6
" " "	178	21	10
" " "	84	20	12
Kansas, May 1955	140	0	0
" " "	140	6	6
Iowa, May 1955	119	37	37
" " "	119	0	6
Georgia, July 1955	149	5	3
Florida, Jan. 1956	117	2	5
Nebraska, Apr. 1956	112	0	0
Avg. 13 Expts. at 6 colleges		11%	10%

(From Wise Burroughs, Iowa State College, June 1956, Inter-regional Livestock Production & Marketing Conference, Ithaca, N.Y.)

Table 5

Average Daily Gains of Steers in Two Feeding Tests

Protein Supplement	1 lb. Soybean Oil Meal	.5 lb. Soybean Oil Meal and 1.25 lbs. Dehydrated Alfalfa	2.5 lbs. Dehydrated Alfalfa	5.0 lbs. Dehydrated Alfalfa
Without Stilbestrol in Ration	2.07	2.30	2.11	2.46
With Stilbestrol in Ration	2.47	2.59	2.64	2.64
% Increase in Gain by Adding Stilbestrol	19.3	12.6	9.5	7.5

(From J. K. Matsushima, University of Nebraska - Nebraska Expt. Sta. Qtrly., Spring 1959)

Table 6

Some Effects of Diethylstilbestrol Pellets on Single Comb White Leghorn Cockerels[a]

Treatment	Birds 14 weeks old				Birds 16 weeks old			
	Diethyl-Stilbestrol Absorbed, mg.	Testes Wt.,g.	Comb Wt.,g.	Leaf Fat Wt.,g.	Diethyl-Stilbestrol Absorbed, mg.	Testes Wt.,g.	Comb Wt.,g.	Leaf Fat Wt.,g.
Control	0	5.67	20.4	28.2	0	7.22	27.4	11.8
Pellet A.	10.1	0.48	2.9	47.6	10.6	0.60	3.5	64.6
Pellet B.	8.5	0.42	3.9	44.5	10.6	0.73	4.6	81.3

[a]From F. W. Lorens, Department of Poultry Husbandry, University of California, Davis, California, in Vitamins & Hormones 12: 235. 1954.

WHERE WE STAND ON SAPONINS

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INTRODUCTION

Present studies on saponins in forages at WRRL began when several investigators reported that alfalfa contained a factor which caused growth inhibition in young chickens when it was fed at high levels. Drs. Cooney and Butts at the Oregon Experiment Station; Dr. H. S. Wilgus at the Colorado Experiment Station; Mr. Burt Heywang at the Southwest Poultry Experiment Station, Glendale, Arizona; and Drs. Lepkovsky and Peterson at the University of California, all agreed that 10 to 20% levels of some alfalfas in chick diets could cause reduced growth.

Lepkovsky and Peterson prepared crude alfalfa extracts which would cause reduced chick growth and which had many characteristics of saponins. They postulated that saponins were responsible for the deleterious effect. Following this disclosure, an active investigation of forage saponins was begun at WRRL. This program has now continued for about eight years.

Chemistry of Legume Saponins

Saponins are soap-like glycosides made up of several sugars such as glucose, galactose, xylose, etc., and a fat-soluble nucleus called a sapogenin. Legume saponins, of which there are several, yield triterpene sapogenins, some of which have proved to be new compounds. Medicagenic acid was isolated from alfalfa and characterized. It was a new compound. Two other sapogenins, Soyasapogenol B and Soyasapogenol C, had previously been found in soybeans and were found in ladino clover and alfalfa. Soyasapogenol B was found also in strawberry clover and birdsfoot trefoil. Lucernic acid has been isolated from alfalfa and partially characterized. The sapogenins have not shown physiological effects when applied to biological systems, possibly because they are insoluble in water but the saponins from alfalfa, ladino clover, and other forage legumes have given marked responses in some instances.

Physiological Effects of Legume Saponins

Alfalfa saponin, isolated by forming an addition product with cholesterol in an aqueous alfalfa extract, hemolyzes red blood cells in 1-60,000 dilution. It also will kill aquatic animals such as snails and fish in 1-1000 dilution. Addition of alfalfa or ladino clover saponins to excised rabbit muscle strips in a physiological saline bath caused cessation of peristalsis. Feeding alfalfa saponins prepared via cholesterol to chicks caused growth inhibition at 0.2 and 0.4% of the diets. Single doses of 25 to 100 g. given to sheep or heifers caused symptoms of ruminant bloat. Autopsy of sheep which died from this disease showed a pronounced hyperemia of the rumen wall. This extreme irritating effect on mucous membrane was confirmed by injecting 25-mg. doses of alfalfa saponins into the small intestine of anesthetized rats. An abrupt

reddening and swelling of the intestine was observed. This effect may occur during ruminant bloat. Also, the rise in blood cholesterol found during ruminant bloat may be caused by the saponins liberated from legume forages.

Alfalfa contains other saponins which fail to form an insoluble complex with cholesterol readily. These compounds which comprise about 70% of the total are suspected to be relatively inactive in causing the pronounced physiological effects detailed above. The evidence for this supposition is indirect because purified total saponin fractions have not been available for testing. However, chick diets which contain as much as 0.4 - 0.5% of total alfalfa saponins cause slight chick growth inhibition comparable to that observed with 0.2% of "cholesteride" saponins. Recent cooperative comparisons between the total saponin content of dehydrated alfalfa and the degree of chick growth inhibition also correlated (Table 1).

Table 1

Effect of Alfalfa Saponin
on Chick Growth

Saponin %	Gain 6 Weeks Grams	Diet Efficiency
0.0	639	0.48
0.1	631	0.48
0.2	556	0.46
0.4	459	0.44

The feeding studies were done by Mr. B. W. Heywang at the Southwest Poultry Experiment Station. He used moderate energy diets and Leghorn chicks. Thus these studies would indicate that the inhibitory fraction may be a constant proportion of the total saponin fraction and that cholesterol may selectively add to the more inhibitory fraction. Whether or not the saponins react with cholesterol in the animal body is yet to be determined.

Detailed studies at this Laboratory with high-energy diets showed that seven different high-quality dehydrated alfalfa meals and two grass meals caused no significant growth inhibition even at 15% of the total diet. Feed efficiency was reduced but this is expected because of the reduced energy. Pathological examination of the birds fed the cholesteride saponin showed no positive symptoms--only reduced size.

These studies prove that <u>some</u> alfalfa meals contain compounds which will reduce chick growth when the amounts fed are high enough. However, as alfalfa is now used in mixed poultry feed the problem is of minor importance.